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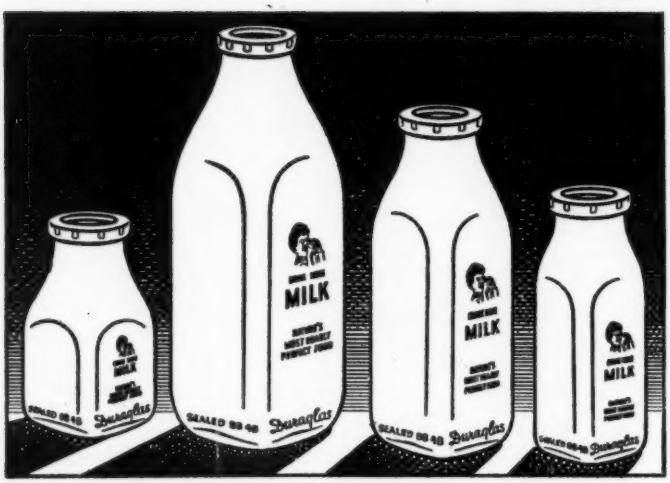

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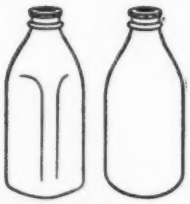

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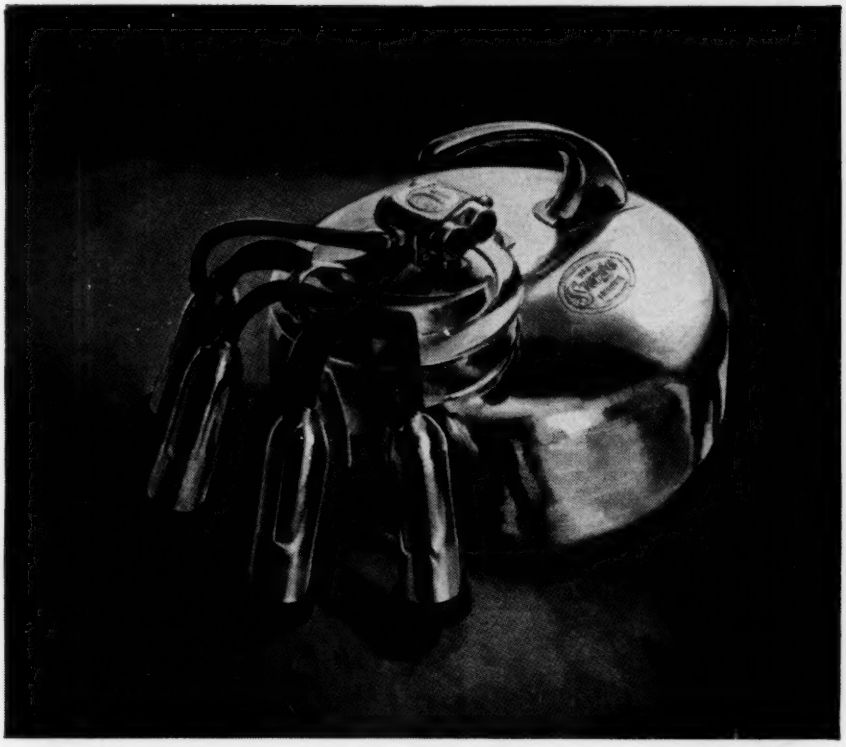

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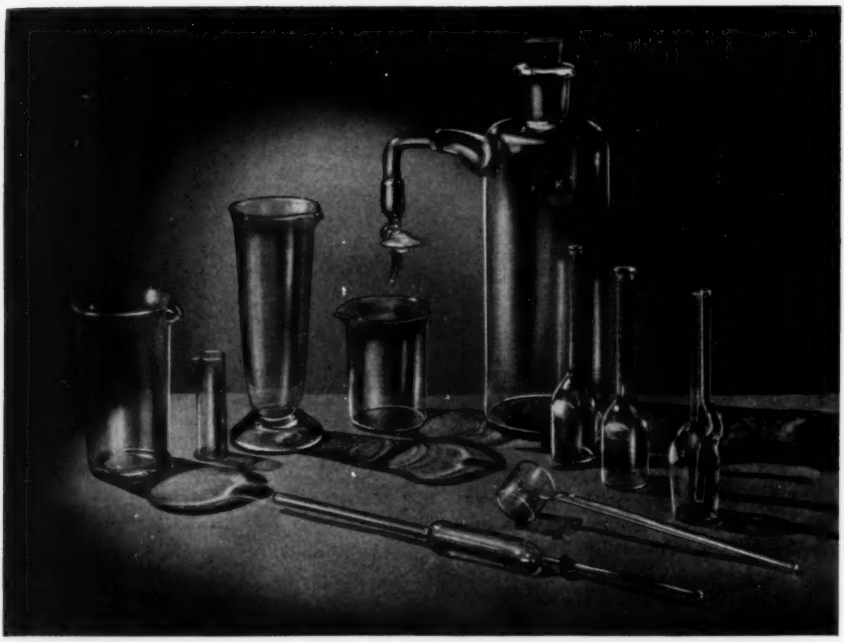

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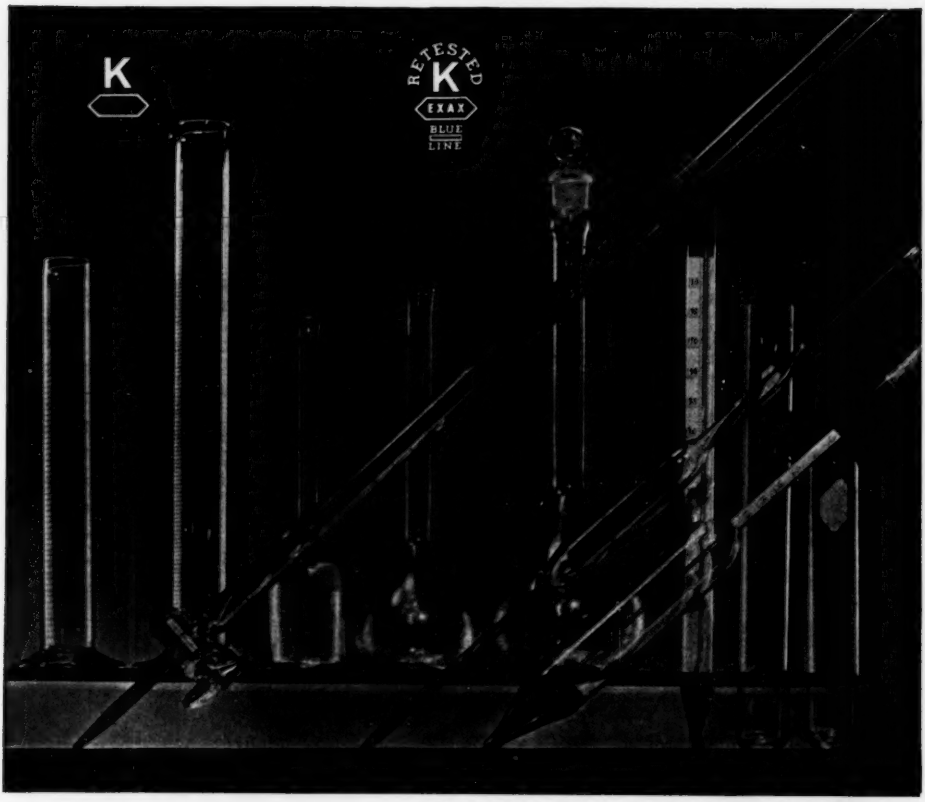

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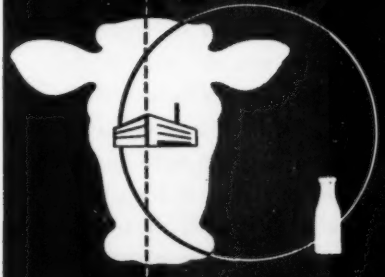

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JOURNAL OF DAIRY SCIENCE

VOLUME XXXII DECEMBER, 1949 NUMBER 12

A NEW LYOPHILIZING APPARATUS

J. W. STULL[1], AND E. O. HERREID

Department of Food Technology, University of Illinois, Urbana

In connection with a research project dealing with some heat labile fractions of milk, it was desired to build a lyophilizing apparatus. The lyophilizing process (drying from the frozen state) was described in detail first by Shackell (10) in 1909. Five years later, Rogers (8) described the design, construction and application of another lyophilizing apparatus. His classic study of the preparation of dried cultures of microorganisms apparently did not arouse much interest until 1935, when two groups of workers (4, 5) published descriptions of lyophilizing apparatus and discusssed their application to the drying of biological substances, including cultures of microorganisms. A summary of the theory of the lyophilizing process is given by Bradish (1) and Bradish *et al.* (2). A review of the recent literature dealing with the construction of lyophilizing apparatus revealed a wide variation in design, size, simplicity and efficiency of operation (1, 2, 3, 6, 7, 9, 11). Without going into detail concerning the theory of the lyophilizing process, it may be said that the construction of the equipment described herein, provides for the optimum conditions of the lyophilizing operation, namely, (a) the maintenance of a high vacuum, (b) short, direct path from the surface of the material to a condenser which is maintained at the temperature of dry ice, and (c) drying from a thin layer of frozen material.

Construction of apparatus. Fig. 1 shows the lyophilizing apparatus. The outer member of a 71/60 standard taper ground glass Pyrex joint was fitted so as to replace the neck of a 3 l. round bottom flask (A). At a point 2.0 cm. above the junction of the neck with the body of the flask, the outer member of a 29/42 standard taper ground glass Pyrex joint was sealed as a side arm into the neck at an angle of 45°.

The condenser was constructed from a glass tube (o.d. 3.0 cm.) which was ring-sealed at the top of the inner member of the 71/60 ground glass joint. The tube was sealed off at the bottom such that there was a clearance of 3.0 cm. between it and the bottom of the flask when the inner member was placed into position.

Four complete units[2] were constructed to be connected to the manifold (B) through which the vacuum was applied by means of a mercury diffusion pump.

Received for publication July 11, 1949.

[1] Now at the University of Arizona, Tucson.

[2] Two units were constructed from 3 l. round bottom flasks and two from 5 l. flasks.

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The manifold was constructed from a 500 ml. round bottom flask. The four inner members of the 29/42 standard taper joints were sealed into the bottom of the flask at an angle of 135° with the neck of the flask. A 2.0 mm. two-way stopcock was sealed into the bottom of the manifold to be used in releasing the vacuum. The vacuum was applied at the top of the manifold.

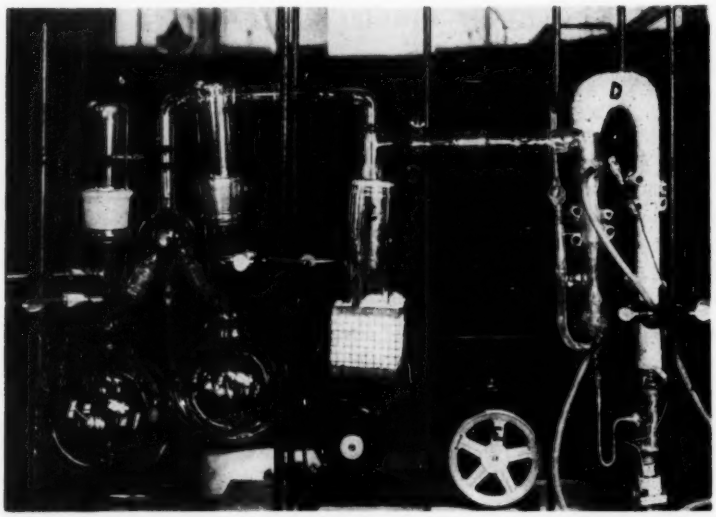

FIG. 1. Assembled apparatus with the vacuum system attached. A—lyophilizing units. B—manifold. C—liquid air trap. D—mercury diffusion pump. E—backing pump.

Operation of the apparatus. The operation of the apparatus is as follows:

(a) Pour the material to be dried into the flask.

(b) Immerse the flask and contents in an acetone-dry ice bath and freeze the material on the inside surface of the flask in a thin, uniform layer by swirling.

(c) Place the condenser into position and connect the assembly to the manifold.

(d) Apply the vacuum.

(e) Place acetone-dry ice coolant in the condenser tube at a level even with or above the side arm of the flask.

(f) Place a fan[3] in a position which will direct a current of air against the flasks.

(g) Drying is complete when the flasks reach room temperature. Release the vacuum, disconnect each assembled unit from the manifold and remove the inner member which holds the moisture in the form of ice.

Operating characteristics. Typical operating results are found in table 1.

[3] A 16-in. ventilating fan was placed within 6 in. of the flasks and operated at full speed.

TABLE 1

Typical results obtained in the operation of the lyophilizing apparatus

Product	Trial no.		
	1	2	3
	Buttermilk	Condensed washed cream buttermilk	Condensed washed cream buttermilk
Wt. of product (*g.*)	230	230	275
Total solids (%)[a]	11.5	10.1	10.1
Wt. of powder (*g.*)	27.3	23.2	27.5
Moisture (%)	2.9	3.5	3.7
Time of operation (*hr.*)	5	3	3
Room temp. (° *C.*)	22–23	28–29	28–29
Size of flask (*l.*)	3	3	5
Mean rate of moisture removal (*g./hr.*)	40.4	69.7	82.2
Vacuum applied (*mm. Hg.*)[b]	0.1	0.1	0.1
Wt. of water removed (*g.*)	201.9	206.0	246.5

[a] Mojonnier Method.
[b] Determined by vacuum arc tester.

In regard to the rate and amount of moisture removal, three points should be emphasized. First, it is important to secure the vacuum reported not only to maintain the maximum rate but also to derive the indicated capacity of the apparatus. The latter factor results from the fact that the capacity of the apparatus is limited by the maximum thickness of the ice formation which can clear the neck of the flask when the condenser is removed. It has been found, therefore, that as the vacuum applied is increased the structure of the ice changes from fine, loosely formed crystals to a dense, compact mass at a pressure of less than 0.1 mm.

The explanation of the variation in the rates of moisture removal in trials 1, 2 and 3 (table 1) is probably found in the second and third points to be considered, namely, the room temperature and the size of the unit. An increase in room temperature of 6° C. resulted in an increase in rate of moisture removal of more than 50 per cent (trial 1 *vs* trial 2). Finally, through an increase in the size of flask from 3 to 5 l. (trial 2 *vs* trial 3), the rate of moisture removal was raised approximately 20 per cent.

CONCLUSIONS

A lyophilizing apparatus is described in which the design, simplicity, and efficiency represent improvements in the types of apparatus available for small scale operation.

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THE EFFECT OF INTERRUPTION OF MILKING ON THE CAROTENE AND VITAMIN A AND PROXIMATE COMPOSITION OF MILK AND ON THE CALCIUM CONTENT OF BLOOD SERUM[1]

D. N. MERCER, H. D. EATON, R. E. JOHNSON, A. A. SPIELMAN, W. N. PLASTRIDGE, L. D. MATTERSON AND L. NEZVESKY

Animal Industries Department and Animal Diseases Department, Storrs Agricultural Experiment Station, Storrs, Connecticut

The interruption of milking has been found by several investigators (1, 3, 4, 8, 12, 13, 14, 16) to result in a marked alteration of the proximate composition of milk. In general, the amount of milk and the per cent lactose were found to have decreased, the per cent protein and ash to have increased, and the per cent fat to be affected inconsistently. During interruption of milking, an increase in the level of lactose in both blood and urine has been reported (1, 12). Although no reports on the effect of interruption of milking on blood serum calcium were found in the literature, blood serum calcium has been observed (10) to increase the third or fourth day postpartum in cows not milked following parturition. The objectives of this study were to determine the effect of interruption of milking for a 10-day period on (a) the carotene, vitamin A, and proximate composition of milk, and (b) the calcium level of blood serum.

EXPERIMENTAL

Animals. A total of 18 cows of the Ayrshire, Guernsey, Holstein and Jersey breeds were used in this experiment during the period March, 1948, through April, 1949. Twice daily milking was interrupted for a 10-day period in 12 of these cows; that is, no milk was removed from the udders of these cows during the 10-day period. The six remaining cows served as controls and were milked twice daily for the entire experimental period. The average number of lactations and the average number days milked postpartum were 1.8 ± 0.9 and 175.7 ± 26.6, respectively, for those cows in which milking was interrupted and 4.0 ± 1.9 and 104.2 ± 50.2 for the controls.

For 4 wk. prior to the interruption of milking and for the experimental period, all cows received roughage on the basis of liveweight. This consisted per 100 lb. of liveweight of 1 lb. of U. S. No. 2 alfalfa hay and 3 lb. of well-matured corn silage. A grain mixture consisting largely of cereal grains and containing 13.5 per cent crude protein was fed according to milk production. To those cows in which milking was interrupted, no grain was fed during the first 8 days of the interrupted milking period. The hay, silage and grain contained on an average 17.71, 1.89, and 0.16 mg. of carotene per lb., respectively, as de-

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[1] This work was supported in part by the Big-Y-Foundation, Norwich, Conn. and Chas. M. Cox Co., Boston, Mass. It is part of a thesis presented by D. N. Mercer to the Graduate School of the University of Connecticut in partial fulfillment of the requirements for the degree of Master of Science.

termined by the method of Moore and Ely (9) as modified by Nelson *et al.* (11).

Samples. Representative samples from the six milkings immediately prior to the 10-day interruption period and from the first six milkings following interruption were obtained from 12 of the cows. Similar samples plus samples during the 10-day period when the treated cows were not milked were obtained from two of the control cows. Milk samples were chilled in the dark at 4° C. and analyses were completed in most instances within 6 days. When analyses could not be completed within 6 days, the samples were quick-frozen and held at −18° C. until analysed.

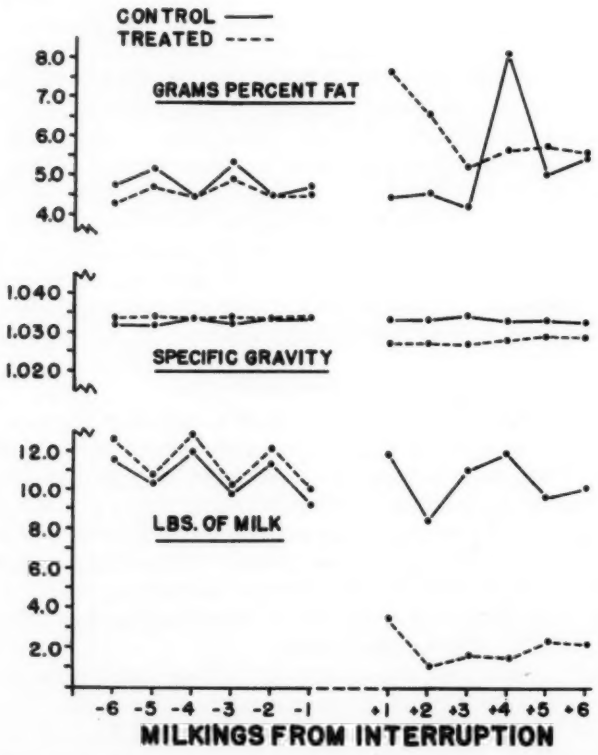

FIG. 1. The effect of interruption of milking on the lb. of milk, specific gravity and per cent fat.

Venous blood samples were obtained daily between 9 and 11 a.m. from four of the cows in which milking was interrupted and from four cows in which milking was not interrupted. The blood was allowed to clot at 4° C., centrifuged within 8 hr., and analyses completed for serum calcium within 72 hr. after collection.

Analyses. The methods used in the analyses of the milk samples were similar to those previously reported (5). Blood serum calcium was determined accord-

TABLE 1

The effect of interruption of milking on the level of calcium in blood serum

Exp. No.	Days															
	Before interruption			During interruption							After interruption					
	3	2	1	1	2	3	5	7	9	10	1	2	3	4	5	7
Treated	(*mg.* % serum calcium)															
1	10.45	10.39	11.11	10.20	11.39	11.23	12.08	11.26	10.64	11.12	10.43	10.40	10.68	10.23	10.45	10.09
2	12.66	11.02	11.28	11.26	13.23	12.77	12.17	10.81	11.53	12.09	10.58	10.98	10.54	10.86	10.74	10.67
3	10.19	10.39	11.20	11.22	12.64	11.08	11.27	11.13	11.00	11.62	10.39	10.82	10.76	10.05	10.21	10.84
4	10.73	10.44	11.42	10.78	10.88	12.36	12.07	10.87	11.39	10.79	10.36	10.25	10.46	10.28	10.38	10.39
$\overline{X}$	11.01	10.56	11.22	10.87	12.04	11.86	11.90	11.02	11.14	11.41	10.44	10.61	10.61	10.36	10.45	10.70
Controls																
5	10.47	11.23	10.31	10.35	10.94[a]	11.55	12.30	11.18	10.97	11.38	10.06	10.34	10.39	10.63	10.63	10.50
6	10.18	10.30	10.36	10.41	9.73[a]	10.42	10.06	9.46	10.41	9.67	10.51	10.55	10.95	10.43	10.59	10.59
7	10.39	10.51	10.73	10.69	10.02[a]	10.35	9.94	10.87	10.46	9.86	9.59	10.47	10.70	10.46	10.62	10.71
8	10.90	10.42	10.55	10.36	10.10[a]	10.44	9.87	10.51	10.26	11.21	10.73	11.59	10.45	10.60	11.04	11.37
$\overline{X}$	10.49	10.62	10.49	10.45	10.20	10.69	10.54	10.51	10.53	10.53	10.22	10.74	10.62	10.53	10.72	10.79

[a] Observation calculated according to missing plot technique (15).

ing to the method of Clark and Collip (2). Standard statistical procedures for the analysis of variance (15) were used to test for differences between treatments.

RESULTS

Data for the mean carotene and vitamin A and proximate composition of the milk for the six milkings prior to interruption and for the six milkings after interruption are given in figs. 1, 2, 3 and 4. The calcium levels in the blood serum of individual cows prior to, during, and after the interruption of milking are contained in table 1. Interruption of milking resulted in a decrease in the per cent lactose, and increases in the per cent carotene, vitamin A, protein, fat, and ash. With the exception of carotene and vitamin A, the total amount of these nutrients secreted was less after interruption than before interruption. The levels of blood serum calcium in those cows in which milking was interrupted were found to increase during the interruption period.

The average amount of milk and specific gravity (fig. 1) of the milk of those cows in which milking was interrupted was significantly less after interruption than prior to interruption. An analysis of the differences of the average values before interruption from the average values after interruption gave highly significant differences ($P < 0.001$) between treatments. Further analyses between average values for the six milkings prior to interruption and similar average values after interruption within treatments showed a similar statistical difference for the group in which milking was interrupted, but no statistical difference was found for the control group.

The average carotene and vitamin A content per 100 ml. of milk or calculated to per gram of fat (fig. 2 and 3) increased markedly after interruption of milking. The differences in the average values before interruption from those after interruption were statistically significant between treatments for vitamin A ($P < 0.01$ for per 100 ml. of milk and $P < 0.05$ for per g. of fat) but not for carotene. Within the group in which milking was interrupted, there was a significant increase ($P < 0.001$) in carotene and vitamin A following interruption. No statistical differences were found for the control group. The trend for carotene after interruption was negative while that for vitamin A was positive. A calculation of the total amount of carotene and vitamin A secreted showed no significant differences between the average values before interruption with those following interruption.

The average protein, fat, and ash content (fig. 1 and 4) increased after interruption, while the average lactose content decreased. The differences between the average values before interruption and after interruption showed significance only for lactose ($P < 0.001$) and for ash ($P < 0.05$). However, a comparison within treatments of the average values before and after interruption gave highly significant differences ($P < 0.01$ in the case of protein and $P < 0.001$ in the case of fat and ash). Both protein and ash showed negative trends after interruption, and lactose a positive trend. Interruption of milking caused significant decreases ($P < 0.01$) in the total amounts of these nutrients.

Although not included in the data presented, aliquot daily samples represent-

ing the fourth, fifth, seventh, and ninth days after interruption were obtained from seven of the cows in which milking was interrupted. The amount of milk, the specific gravity, the per cent fat and the per cent protein had by the ninth day returned to the same average levels as those found before interruption of milking. Such is not the case, however, with the per cent lactose, the per cent ash and the carotene and vitamin A content expressed as micrograms per cent or per gram of fat.

The levels of blood serum calcium (table 1) were increased by interruption of milking. During the interruption of milking the average serum calcium levels were higher ($P < 0.05$) in the interrupted milking group than in the controls. There were no treatment differences prior to and after interruption of milking.

DISCUSSION

Investigation in the field of interrupted milking prior to this study has indicated that, when the removal of all or part of the milk from the mammary

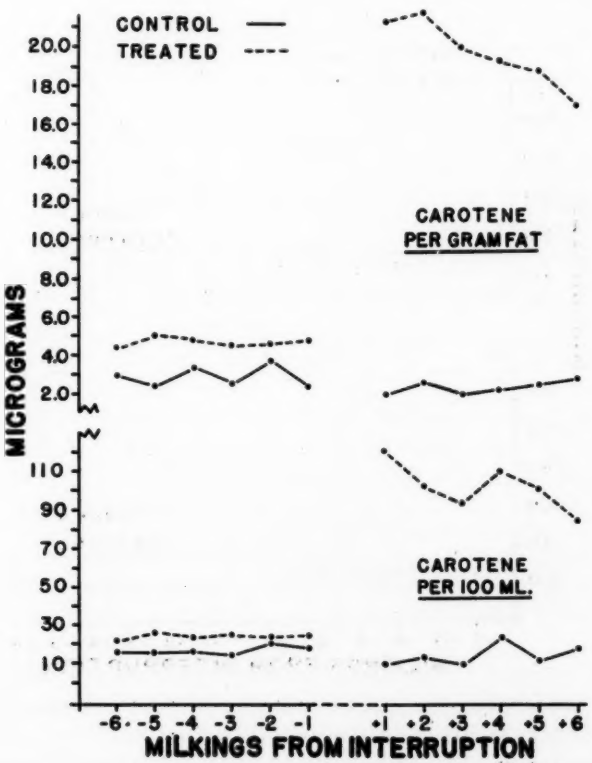

FIG. 2. The effect of interruption of milking on the carotene content of milk.

gland has been interrupted for a period of time and then resumed, changes occur in the concentration of the proximate constituents. These changes have been attributed to resorption of certain of these constituents. Previous work has utilized the individual cow's udder as an experimental unit and a control unit. The possibility of stimulatory effects resulting in higher intramammary pressures than in the non-stimulated udder probably were experienced, if the "let-down" mechanism of Ely and Peterson (7) is accepted.

The effect of interruption of milking on the concentration of the proximate constituents as reported herein is essentially in agreement with the data in the literature (1, 3, 4, 8, 12, 13, 14, 16). Examination of the total amount of these nutrients secreted after interruption would tend to support the view that resorption does take place upon interruption of milking. A more critical experiment certainly is indicated before this view can be accepted fully for all of the proximate constituents and their component parts.

In the case of carotene and vitamin A, the increase in concentration was marked, both when expressed as γ per 100 ml. of milk and γ per gram of fat. Of further interest is the positive trend in the concentration of vitamin A fol-

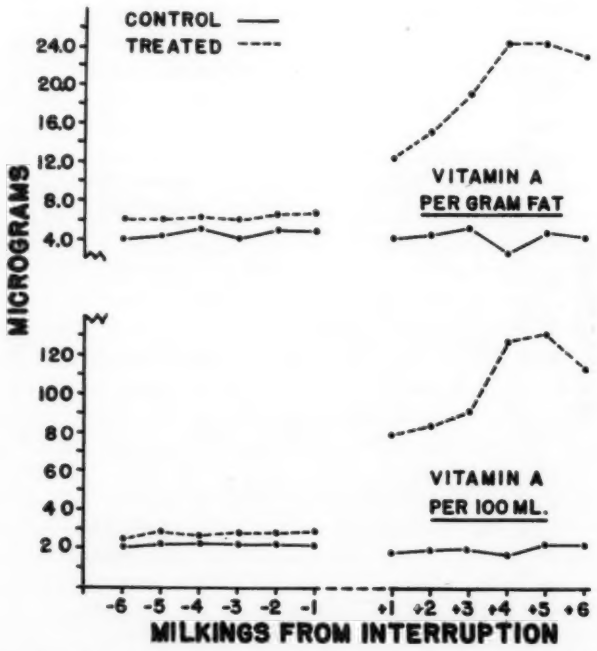

FIG. 3. The effect of interruption of milking on the vitamin A content of milk.

lowing interruption of milking which suggests that, at least under these particular conditions, the secretion of vitamin A may be independent of that of fat. The

absence of significant differences between the total amount of both carotene and vitamin A secreted before and after interruption of milking is in contrast to that found for the proximate constituents, where a marked decrease occurred.

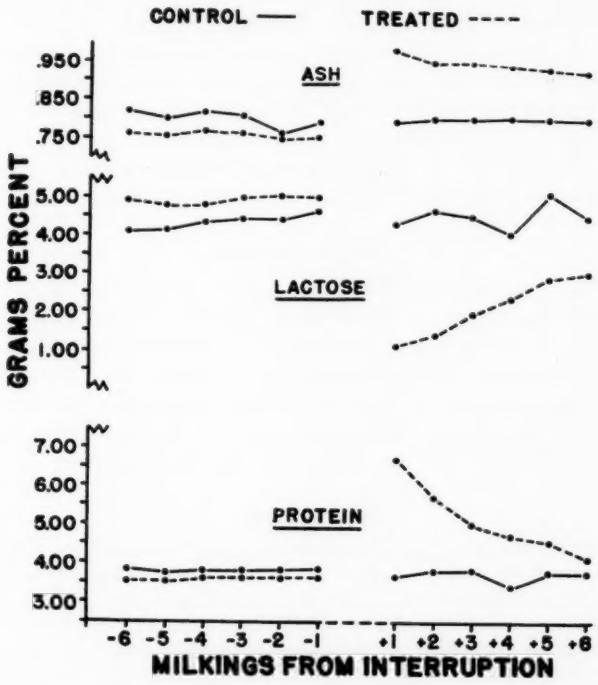

FIG. 4. The effect of interruption of milking on the per cent protein, per cent lactose, and per cent ash of milk.

Porcher (12), and Brown *et al.* (1) have pointed out that interruption of milking will result in a secretion similar to that of colostrum. The data presented in this paper would not support such a view. Colostrum from the first milking postpartum of cows fed a ration similar to that in this experiment (6) had concentrations of lactose, fat, and ash, only, which approached those values reported in the data in this experiment. In colostrum the average grams per cent protein was 12.2 and the average micrograms per cent carotene and vitamin A were 272 and 268. These values were two-fold greater than those found after interruption of milking.

The increase in blood serum calcium during the interruption of milking is similar to the report of Neidermeier and Smith (10), who found in cows not milked postpartum a similar rise 3 to 4 days after parturition. Whether this rise in serum calcium is due to resorption of calcium or to hormonal effects needs investigation.

SUMMARY

The effect of interruption of milking for a 10-day period during lactation on the carotene, vitamin A, and proximate composition of milk and on the calcium level of blood serum has been studied for 18 cows. The data indicate that interruption of milking results in significant increases in the concentration of carotene and vitamin A. With the exception of lactose, the proximate constituents also increased in concentration; lactose content decreased. The total amount of these nutrients secreted for the first 3 days after interruption was significantly lower for the proximate constituents, but no appreciable differences were noted for carotene and vitamin A. Interruption of milking, resulted in a significant elevation of blood serum calcium during the period of interruption.

ACKNOWLEDGMENTS

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THE VALUE OF MILK REPLACEMENTS IN THE RATIONS OF DAIRY CALVES[1, 2]

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INTRODUCTION

Numerous attempts have been made to find a method of raising young dairy calves on a minimum amount of whole milk. Several investigators (1, 2, 3, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17) have demonstrated that calves can be raised on limited quantities of milk.

The relative cost of fluid milk in some areas has encouraged the sale and use of various products in supplementing or replacing whole milk. Some of the materials used are dried skimmilk, dried whey, distillers dried solubles, soluble blood flour, oat flour, fish meal, vitamin supplements and other animal and vegetable by-products. Shoptau (18) found that soybean flour was not a satisfactory substitute for milk. Wiese *et al.* (21) concluded that a synthetic milk preparation must be supplemented with riboflavin to give normal growth. Trimberger (20) has reported that distillers dried solubles can be used to replace half of the milk normally fed to calves at 3 wk. of age. Gullickson *et al.* (4) observed that calves fed vegetable oils or animal fats did not gain weight or appear as thrifty as calves fed whole milk.

The purpose of this investigation was to obtain additional information relative to the value of mixtures of some commonly used animal and vegetable products when fed to dairy calves as replacements for milk.

EXPERIMENTAL PROCEDURE

Fifty Holstein male calves obtained from state institutional herds, were divided into five comparable groups on the basis of body weight, height at the withers and chest circumference. The calves were put on the experiment not later than the fourth day following birth. Groups I, II, III and IV were fed the replacement formulae shown in table 1. The remaining ten calves constituted the control group and were fed 300 lb. of whole milk including colostrum. All calves were fed from open pails placed in the concentrate feed box located 10 inches above the floor of the pen.

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[2] The data contained in this publication are from a thesis to be submitted by the senior author to the Graduate School of The Pennsylvania State College in partial fulfillment of the requirements for the degree of Doctor of philosophy.

TABLE 1

Milk replacement formulae

Ingredient	I	II	III	IV
	(*lb.*)	(*lb.*)	(*lb.*)	(*lb.*)
Dried skimmilk	40	50	50	30
Dried whey	20	10	10	10
Ground beet pulp	20	10	.....	10
Corn distiller's dried solubles	.....	10	10	10
Blood flour	.....	.....	10	10
Fish meal	.....	.....	.....	10
Dextrose	7.75	7.75	7.75	7.75
Oat flour	5.00	5.00	5.00	5.00
Brewer's dried yeast	4.90	4.90	4.90	4.90
Irradiated yeast (9F)	0.10	0.10	0.10	0.10
Stabilized Vitamin A feed[b]	2.20	2.20	2.20	2.20
Minerals[c]	0.042	0.042	0.042	0.042
Daily allowance	1.0	1.0	1.0	1.0
Est. daily intake of dicalcium phosphate (*lb.*)	0.1846	0.2195	0.301	0.2888
Est. daily intake of Ca (*g.*)	3.80	3.83	3.90	5.36
Est. daily intake of P (*g.*)	3.05	3.79	4.04	4.62
Cost[a] per calf to 8 wk. of age	$4.69	$5.12	$5.76	$5.01

[a] Cost based on retail feed prices in Aug., 1948.
[b] 220,000 USP units of vitamin A/lb.
[c] Mineral mixture: Ferric citrate ($FeC_6H_5O_7 \cdot 3H_2O$) 56.57%
Cupric sulfate ($CuSO_4 \cdot 5H_2O$) 19.73%
Manganese sulfate ($MnSO_4 \cdot 4H_2O$) 21.59%
Cobalt chloride ($CoCl_2 \cdot 6H_2O$) 2.11%

The calves in groups I, II, III and IV were fed the mixtures at 100° F. according to the following schedule:

Birth to 4th day
Dam's milk
5th to 7th day
2.5 lb. whole milk, 0.25 lb. milk replacement, 2 lb. water[3]
8th to 10th day
1 lb. whole milk, 0.5 lb. milk replacement, 4 lb. water[3]
11th to 35th day
0.5 lb. milk replacement, 5 lb. water[3]
36th to 49th day
0.5 lb. milk replacement, 6 lb. water[3]
50th to 56th day
0.5 lb. milk replacement, 6 lb. water (once daily).

The calves in group V were fed whole milk which averaged about 3.8 per cent fat according to the following schedule: birth through 10th day—8 lb. per day, 11th through 24th day—10 lb. per day, 25th through 28th day—8 lb. per day and 29th through 36th day—6 lb. per day.

The calves of all groups were fed a good quality alfalfa hay *ad libitum* from birth to the conclusion of the 16-wk. trial. Each calf was provided with calf starter *ad libitum* until each was consuming the maximum of 6 lb. daily. The

[3] Twice daily.

calf starter was prepared as follows: yellow corn meal 406.5 lb., wheat bran 300 lb., crushed oats 400 lb., linseed oil meal 140 lb., soybean oil meal 280 lb., dehydrated alfalfa meal 140 lb., cane molasses 100 lb., dried skimmilk 100 lb., dried corn distiller's solubles 100 lb., irradiated yeast (9F) 0.5 lb., dicalcium phosphate 10 lb., ground limestone 10 lb., iodized salt 10 lb., and Vitamin A feeding oil 3 lb. (2,724,000 USP units of A per pound).

The barn was artificially lighted and ventilated, and thermostatically maintained at a temperature of 65° F. by means of steam heat. Each calf was placed in an individual pen which was equipped with a salt block, water bowl, concentrate feed box and hay manger. The calves were placed at random throughout the barn so as to prevent positional effects. Growth measurements were taken by the same person each week at the same time and in the same order with respect to the body weight, height at the withers and chest circumference. Daily observations of the conditions of the feces of each calf were made by the same person throughout the trial. Photographs of each calf were taken at birth, 4, 8, 12 and 16 wk. of age. Upon arrival each calf was given orally, 5 g. of sulfathalidine and an additional 5 g. at each of the next three successive feedings.

EXPERIMENTAL RESULTS

TABLE 2

Summary of growth[a] and cost data

Group	Body weight		Withers height		Chest cir.		Cost[b]
	8 wk.	16 wk.	8 wk.	16 wk.	8 wk.	16 wk.	16 wk.
I	0.33	1.07	0.07	0.10	0.02	0.07	0.20
II	0.78	1.41	0.13	0.15	0.06	0.09	0.18
III	0.84	1.35	0.10	0.14	0.07	0.09	0.19
IV	0.89	1.32	0.12	0.14	0.08	0.09	0.19
V	0.90	1.36	0.12	0.14	0.06	0.09	0.22

[a] Expressed as mean daily gains.
[b] Based on retail feed prices in Aug., 1948 per lb. gain.

The summary of the growth data for the 16-wk. trial is presented in table 2. It would appear from this summary that the calves of group II were slightly superior to the other groups, but when the data were analyzed statistically according to the methods of Snedecor (19) there were no significant differences between groups II, III, IV and V in terms of body weight, height at the withers and chest circumference. However, group I was significantly poorer than all other groups on the same basis. The cost per lb. of gain in body weight was somewhat lower for groups II, III and IV than for V. The high cost of fluid milk, even though fed in limited amounts, was the reason for the higher cost in group V. Gain was less costly in group I as compared to group V, but rate of gain was considerably less also.

The only loss was one calf in group I which died at 30 days of age. The individuals in this group suffered frequent and prolonged scouring until about 30 days of age, with two calves being unable to stand on their feet for several days

FIG. 1. Calf 418, a typical individual in group I at 4 wk. of age.

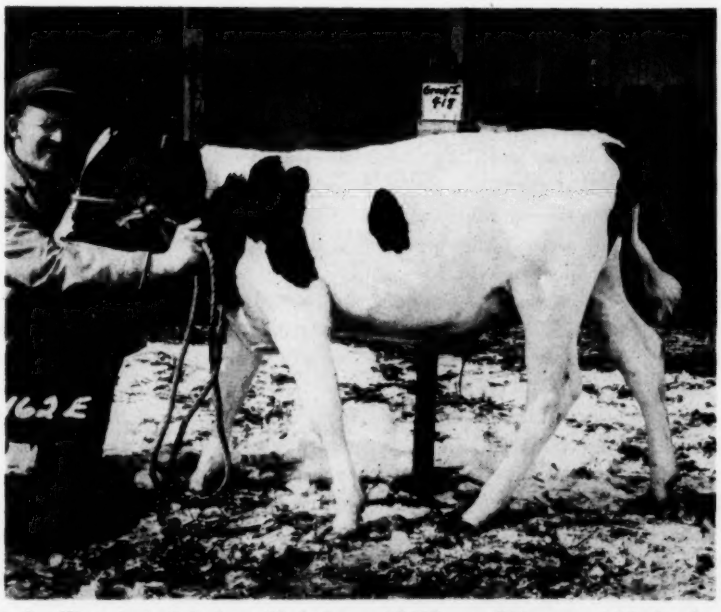

FIG. 2. The extent of the loss of hair from calf 418 can still be seen at 16 wk. of age, but complete recovery is apparent.

at that age. All calves in group I incurred considerable hair loss over the entire body, muscular incoordination and weakness, profuse lacrimation and papilladema but maintained their appetites even when unable to stand. Fig. 1 is a photograph of calf no. 418, a representative calf of group I, at 4 wk. of age, while fig. 2 is the same calf at 16 wk. of age. The hair loss began on the 18th to 21st day of age and continued until the calves began to ruminate at an average of 35 days of age, at which time the hair loss stopped and new hair began to grow in. Three calves in group II suffered some loss of hair about the forehead and one calf in group IV suffered considerable loss of hair over the entire body. There was no hair loss in groups III and V.

The amounts of hay and grain presented are those actually consumed, as all refusals were weighed back. The average consumption of the calf starter was similar in all groups except group I. This group consumed an average of 301 lb. of starter and the other four groups varied from 351 lb. to 363 lb. Average hay consumption per calf for groups II, IV and V were 150, 144 and 151 lb., respectively; calves in group I each consumed an average of 112 lb. and calves in group III each 172 lb.

Scouring was not a problem in any of the groups with the exception of group I. When a scouring condition persisted for more than 24 hr., a 10 g. dose of sulfathalidine was given and another 5 g. at each of the next two successive feedings. The drug was administered orally in 5 g. capsules and effectively controlled all cases except those occurring in group I. As high as 40 g. were given to calves in group I without any apparent relief from the condition. The teeth of four of the calves in group I were loose and greatly discolored with red, tender gums. Some of the calves suffered tongues swollen so badly that swallowing was difficult, even though the calf was hungry. The mouth was very tender, making it painful to work the trip in the water bowls; the water bowls had to be operated manually by the feeder until the condition cleared up.

The feces of all of the calves on the replacement formulae were very dark and rather soft until a considerable amount of hay and starter were being ingested, at which time they were similar in all respects to the feces of the milk-fed calves.

The general appearance of the animals in groups I, II and IV was not as satisfactory as the calves in groups III and V; however, at 60 days of age there was very little difference in the groups. At the end of the 16-wk. trial several of the calves from group I were comparable to the other groups in all respects.

Palatability was not a problem with any of the formulae, as calves 4 days old easily were taught to take the mixtures from the pail. Mix I had a greater tendency to settle out than the others, especially when the very young calves were slow in consuming the mixture. The other mixes went into suspension very readily and remained so until the calf had consumed the entire amount. One animal in group III persistently refused to take the replacement unless aided by the feeder.

In an effort to correct the symptoms occurring in group I additional calves similarly were fed and managed but received in addition daily by oral administration the following; calf 411A—50 mg. ascorbic acid, calf 411B—10 mg. biotin,

calf 411C—0.2 lb. vitamin free casein, calf 411P—20 mg. calcium pantothenate, calf 411R—5 mg. riboflavin, calf 481—10,000 USP units of vitamin A, calf 480—2.7 mg. of 70 per cent choline chloride, calf 482—0.7 gm. l-cystine, calf 483—3.5 gm. methionine, and calf 485—received the latter three in combination in identical amounts daily. These supplements failed to effect the general pattern of hair loss and other symptoms observed in group I.

Sixteen additional calves have been fed a milk replacement similar to that fed to group III except that it contained 0.22 lb. of stabilized vitamin A feed (2,274,000 USP units per lb.) which replaced 2.20 lb. of stabilized vitamin A feed (220,000 USP units of vitamin A) and 2.50 lb. of dicalcium phosphate in addition. Similar response in growth and general appearance was obtained. This modified formula is now being used in a field trial with a relatively large number of calves and is giving satisfactory results.

SUMMARY

1. A milk replacement formula is presented which produced calves equal in rates of growth and general appearance to milk fed controls under the conditions of these experiments.

2. A replacement containing 20 per cent beet pulp was found to produce certain deleterious effects, under the conditions of this trial. These symptoms were not prevented by the oral administration of ascorbic acid, biotin, vitamin free casein, calcium pantothenate, riboflavin, choline-chloride, l-cystine, methionine or vitamin A.

The cooperation of V. A. Houston and C. R. Barber of the Pennsylvania Department of Welfare in providing the calves used in this project is sincerely appreciated. This was a cooperative project with the Department of Animal Husbandry of Cornell University.

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THE USE OF *CANDIDA LIPOLYTICA* CULTURES IN THE MANUFACTURE OF BLUE CHEESE FROM PASTEURIZED HOMOGENIZED MILK[1]

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The pasteurization of milk for the manufacture of blue cheese has received increasing interest since the enactment of various state laws requiring pasteurization or minimum curing periods for cheese. Pasteurization destroys the normal milk lipase which is responsible for much of the fat breakdown required in the development of the flavor of blue cheese. One logical solution to the problem is the substitution of another lipase for the milk lipase. The present study is an attempt to substitute, for the lipase of milk, the lipases produced in the cheese by certain microorganisms added as pure cultures to the milk from which cheese is made.

HISTORICAL

The manufacture of blue cheese from pasteurized milk was mentioned by Goss, Nielsen and Mortensen (8) in 1935. Cheese made from pasteurized milk did not develop as much surface growth of molds and bacteria, did not have as much flavor and did not become as sweet during curing as did the cheese made from raw milk. Lane and Hammer (11) found that the flavor and color of cheese made from pasteurized homogenized milk were inferior to those of cheese made from raw homogenized milk.

Irvine (10) reported that the addition of 0.5 to 1.0 g. of lipase preparation to 100 lb. of raw milk accelerated fat hydrolysis but always gave a bitter flavor in blue cheese. The lipase used was identified as steapsin (13). Coulter and Combs (4) found that this enzyme would give the same amount of flavor in 5 months' curing that had been obtained previously in 12 months' curing of raw milk cheese; however, the bitter flavor was present. They made several lots of blue cheese from pasteurized milk to which 2 g. of steapsin per 100 lb. of milk had been added. These lots of cheese were not inoculated with mold and were not pierced but they had the rank flavor of butyric acid to the exclusion of all other flavors up to 15 months. The volatile acid values were very high on these lots of cheese.

By adding controlled amounts of desiccated sheep mammary tissue to blue cheese curd made from pasteurized milk, Lane and Hammer (12) were able to make a cheese which would ripen in the same length of time required for cheese made from raw milk containing homogenized fat. Babel and Hammer (1) found that addition of steapsin to the curd or to the milk resulted in cheese having a bitter flavor and somewhat gray color. Desiccated mammary tissue produced cheese having more flavor than the control cheese, and the cheese, if made from pasteurized homogenized milk, were quite satisfactory after ripening for 3 months.

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Cheese made from pasteurized milk, to which various enzymes had been added, were improved by homogenization of the milk.

The use of the cell-free extract of *Mycotorula lipolytica* (now termed *Candida lipolytica*) in pasteurized homogenized milk for blue cheese already has been reported by Peters and Nelson (14).

The lipolytic activity of a number of microorganisms has been investigated. The lipolytic activity of *Pseudomonas fragi* was studied by Hussong *et al.* (9) by Fouts (5). *Achromobacter lipolyticum* was found by Collins (3) to produce rancidity in butterfat, corn oil and tributyrin and tallowiness in olive oil and triolein. Fouts (5, 6, 7), in studying the effect of the growth of organisms on the acidity of the fat in cream and butter, found that *Penicillium roqueforti, Oospora lactis, M. lipolytica, A. lipolyticum, Alcaligenes lipolyticus* and *Pseudomonas fluorescens,* but not *P. fragi,* caused increases in the acid numbers of the fat when inoculated into sterilized cream. *M. lipolytica* was the only organism in the group which showed increased growth in the presence of butter culture organisms. Of this group of organisms, *M. lipolytica* and *A. lipolyticus* were found to give the greatest increases in the total volatile acidity of butterfat.

EXPERIMENTAL

The milk used was normal mixed herd milk, pasteurized at 143–147° F. for 30 min., cooled to about 130° F., homogenized at 1,700 lb. pressure and cooled to 40–45° F. The milk was made into cheese immediately or was held below 40° F. until the following day. The milk in the vat was heated to 89–90° F. and 1 per cent of lactic culture was added. After 30 min. ripening, the milk was set with rennet used at the rate of 90 ml. per 1,000 lb. of milk in lots 4 to 51 and 130 ml. per 1,000 lb. of milk in all other lots. The curd was cut with 0.5 inch curd knives 70 min. after rennet addition. The cut curd was allowed to stand for about 30 min. and then was stirred gently every 20 to 30 min. until firm enough to hoop. Heat was applied to the jacket of the vat at the stirring time, whenever necessary to keep the temperature at 90° F. inside the vat. The curd was firm enough to hoop at 2.0 to 2.5 hr. from the time it was cut, and the whey acidity usually had increased 0.04 to 0.06 per cent. One per cent salt and 0.01 per cent mold powder were added to the curd at the time of hooping. Dry salt was rubbed on the surface of the cheese every day for 4 or 5 days, until 5 per cent salt had been used. The day after salting was completed, the cheese of lots 4 to 119 were dipped in flexible cheese coating and pierced about 50 times with a wire needle, 0.1 inch in diameter. All other lots were pierced but not coated. The cheese was cured at 48 to 52° F. at a relative humidity of 90 to 95 per cent.

Each trial consisted of a control lot of cheese and three lots in which one factor was varied, all made from separate 115-lb. portions of the same milk. The lots of cheese were scored at various intervals for mold growth, flavor development and defects in flavor. Each item had a range of score from 0 to 10 in which 10 was the most perfect score. This should be remembered when considering the defect score, because the higher the defect score, the less serious was the defect. The total volatile acidity of the cheese was determined by the method of Lane

and Hammer (11). Fat acidity was determined by the method of Breazeale and Bird (2) after the fat was obtained and purified by the method of Lane and Hammer (11).

Preliminary trials were made to determine the species of microorganism, the type of culture and the amount of culture that should be used to improve the flavor of blue cheese made from pasteurized milk. Cultures of each of the four lipolytic microorganisms, *Candida lipolytica* (strain 846), *Alcaligenes lipolyticus, Achromobacter lipolyticum* and *Pseudomonas fragi* were prepared by inoculating three portions of 18 per cent sterilized homogenized cream with the organisms and incubating one at 30° C. for 24 hr. and two at 30° C. for 48 hr. One of the 48-hr. cultures was sterilized in the autoclave at 15 lb. pressure for 30 min. before it was used in milk for cheese. The cultures were added to milk at the rate of 555 g. (1.2 per cent) per 100 lb. of milk. That amount of cream contained 100 g. of butterfat. The total volatile acidities, fat acidities, scores and comments for the cheese made from milk to which these cultures were added are presented in table 1. Of the four organisms used, *C. lipolytica* gave the largest and most consistent increase in fat acidity and total volatile acidity. The flavor scores for the lots of cheese made with this culture were not high, possibly due to the deficiency of mold growth, but the increase in flavor score over that of the control cheese was greatest for the cheese made with this culture. It was the only culture criticized for producing excess fatty acids in the cheese, which indicated that 1.2 per cent of an active 48-hr. culture of this organism was too much to use. The sterilized 48-hr. culture did not improve the cheese.

Based upon the results of this experiment, another trial was made in which different amounts of a culture of each of the four organisms grown for 48 hr. at 30° C. in sterile homogenized milk were added to each of three lots of milk for cheese. The amounts of culture added and the results obtained are presented in table 2. The improvement of the cheese by the use of a culture of *C. lipolytica* is shown more conclusively by these results. It is the only organism of the four used that increased the total volatile acidity and flavor score appreciably. The use of this culture, at all percentages tried, gave the highest total volatile acidities and flavor scores at 12 wk. of any of the cultures used. The cultures of *C. lipolytica* prepared in homogenized milk appeared to have improved the cheese as much as those prepared in homogenized 18 per cent cream. Fat acidity determinations were discontinued because of the difficulty encountered in extracting the fat and because the values did not appear to be as closely related to flavor scores as were the total volatile acidity values.

As *C. lipolytica* was the only organism of the four used in preliminary studies that consistently improved the flavor and increased the volatile acidity values of blue cheese made from pasteurized homogenized milk, this organism was studied further to determine the effect of various strains of the organism upon the ripening of the cheese. Cultures of eleven strains of the organism from various sources were grown in sterilized homogenized milk for 48 hr. at 30° C., and were added to milk for blue cheese at the rate of 0.3 per cent. Second and third trials were made with some of the strains which gave good results in the first trials.

TABLE 1

The effect of the addition of various cultures of four lipolytic organisms to pasteurized homogenized milk for blue cheese manufacture

Lot no.	Culture in 18% cream incubated at 30° C. Culture used	Age	Total volatile acidity[a]		Fat acidity[b]		Score						Defect comment for cheese 12 wk. old
							Mold		Flavor		Defect		
			4 wk.	12 wk.	4 wk.	12 wk.	4 wk.	12 wk.	4 wk.	12 wk.	4 wk.	12 wk.	
		(*hr.*)											
32	None	.....	7.80	7.60	9.05	2.68	2.5	1.5	1.5	2.0	4.5	1.5	Sour, yeasty, musty
33	*C. lipolytica*	48[c]	5.00	12.00	4.03	7.58	5.0	2.0	5.0	2.0	7.0	1.5	Musty, yeasty (invasion)
34	*C. lipolytica*	24	7.00	13.50	7.45	8.68	2.0	2.5	4.0	4.0	4.5	4.0	Musty, sl. sour, yeasty
35	*C. lipolytica*	48	11.40	26.00	18.00	23.40	4.5	3.5	3.5	3.0	3.0	5.0	Excessive fatty acids
44	None	.....	5.80	15.00	4.58	8.85	5.5	5.0	4.0	5.5	7.0	4.0	Musty, yeasty
45	*A. lipolyticus*	48[c]	6.00	15.30	2.59	18.63	5.0	7.0	5.0	6.0	6.0	3.5	Unnatural, musty, yeasty
46	*A. lipolyticus*	24	6.30	16.80	2.40	26.30	7.0	5.5	5.5	6.5	7.0	3.0	Unnatural, musty, yeasty
47	*A. lipolyticus*	48	5.60	16.30	2.52	10.12	7.5	7.5	6.0	7.0	7.0	2.5	Unnatural, sour, musty, yeasty
36	None	.....	7.00	13.36	5.74	9.23	3.0	3.5	3.0	5.0	4.0	7.0	Yeasty, sl. unclean, green
37	*A. lipolyticum*	48[c]	8.00	15.60	6.12	26.38	2.5	5.0	3.0	7.0	3.0	8.0	Sl. musty, sl. unclean
38	*A. lipolyticum*	24	7.70	15.00	5.26	10.33	5.0	6.0	5.0	7.5	6.5	8.0	Sl. musty, sl. unclean
39	*A. lipolyticum*	48	7.00	20.60	5.88	16.58	8.0	4.5	4.5	6.0	8.0	5.0	Very musty, sl. unclean
40	None	.....	6.90	15.00	1.40	8.18	5.0	6.0	2.5	5.0	6.0	6.0	Sour, musty
41	*P. fragi*	48[c]	6.70	15.30	4.82	17.48	5.5	7.0	3.0	4.0	5 0	4.5	Musty, cheddar
42	*P. fragi*	24	7.00	16.80	5.14	11.98	3.5	5.0	4.5	4.0	8.5	2.0	Musty, unclean
43	*P. fragi*	48	6.70	16.30	4.50	14.22	3.0	6.0	6.0	5.5	9.0	5.0	Musty, unclean

[a] Total volatile acidity expressed as ml. 0.1 *N* acid/200 g. cheese.
[b] Fat acidity expressed as ml. 0.1 *N* acid/10 g. fat.
[c] Culture was sterilized after incubation before addition to milk for cheese.

TABLE 2

The effect of the addition of varying amounts of homogenized milk culture of lipolytic organisms to pasteurized homogenized milk for blue cheese manufacture

Lot no.	Culture used	Culture added	Total volatile acidity[a]		Score						Defect comments for cheese 12 wk. old
					Mold		Flavor		Defect		
			4 wk.	12 wk.	4 wk.	12 wk.	4 wk.	12 wk.	4 wk.	12 wk.	
		(%)									
56	None	.....	6.20	19.50	8.0	4.5	3.5	4.0	3.0	3.5	Sour, unclean
57	*C. lipolytica*	0.1	12.70	30.20	6.0	7.0	6.0	6.0	8.0	4.5	Unclean
58	*C. lipolytica*	0.3	8.60	35.50	8.0	5.0	7.0	7.0	8.0	6.5	Sl. unclean
59	*C. lipolytica*	1.0	17.60	40.90	6.5	6.5	6.5	8.0	7.5	7.5	Sl. unclean (invasion)
60	None	.....	8.00	24.00	3.0	6.5	2.0	4.5	2.0	6.0	Sour, salty, lacking
61	*A. lipolyticus*	0.1	7.40	19.00	5.0	7.0	3.0	5.0	3.0	6.0	Lacking
62	*A. lipolyticus*	0.3	7.00	18.00	3.0	4.0	3.5	4.0	3.5	6.0	Sour, lacking
63	*A. lipolyticus*	1.0	6.80	18.20	3.0	4.5	4.0	4.0	4.0	6.0	Sour, lacking
64	None	.....	5.50	7.80	7.0	6.5	4.0	4.5	3.0	3.5	Unnatural
65	*A. lipolyticum*	0.1	6.00	8.00	8.0	7.5	5.5	5.0	7.0	5.0	Unnatural
66	*A. lipolyticum*	0.3	5.40	8.00	4.5	7.5	3.0	5.5	5.0	6.0	Unnatural
67	*A. lipolyticum*	1.0	5.50	6.00	6.0	6.5	3.0	6.0	4.5	4.0	Unnatural, yeasty
68	None	.....	6.60	8.50	3.0	7.0	3.0	3.5	2.5	3.5	Unnatural, sour, yeasty
69	*P. fragi*	0.1	7.10	7.70	5.5	5.0	3.5	5.0	4.0	5.0	Unnatural, sour, yeasty
70	*P. fragi*	0.3	5.90	6.60	3.5	4.5	5.0	4.0	5.5	4.5	Unnatural, sour
71	*P. fragi*	1.0	6.00	8.50	4.5	6.5	4.5	5.0	5.0	4.0	Unnatural, sour

[a] Total volatile acidity expressed as ml. 0.1 *N* acid/200 g. cheese.

TABLE 3

The effect of the addition of 0.3 per cent culture of various strains of Candida lipolytica *to pasteurized homogenized milk for blue cheese manufacture*

Lot no.	Strain no.	Total volatile acidity[a]		Score						Defect comment for cheese 12 wk. old
				Mold		Flavor		Defect		
		4 wk.	12 wk.	4 wk.	12 wk.	4 wk.	12 wk.	4 wk.	12 wk.	
76	.....	7.40	22.00	6.5	7.0	4.0	3.5	7.0	4.0	Nutty, musty
77	47	6.50	35.00	6.5	8.5	5.5	5.5	7.0	6.5	Nutty
78	57	5.60	24.50	7.0	8.0	5.0	5.0	7.0	7.5	Sl. nutty
79	100	9.00	25.70	6.0	6.0	6.0	4.5	6.0	5.5	Sour, nutty
80	.....	7.30	27.00	7.0	9.0	4.0	3.5	5.0	3.0	Unclean, cowy, sour
81	438	8.60	27.20	7.0	7.0	5.0	5.5	4.0	5.0	Unclean
82	839	19.30	51.40	5.5	5.5	7.5	8.0	8.0	7.5	Sl. excessive volatile acids
83	840	8.30	33.20	7.0	9.0	5.0	6.0	5.0	6.0	Sl. unclean
84	.....	6.80	35.00	5.5	7.5	4.0	4.0	7.5	4.5	Nutty, burned, unnatural
85	843	20.50	50.40	4.0	7.5	7.0	8.0	7.5	7.5	
86	845	8.50	36.80	5.0	7.5	3.5	5.5	4.5	5.0	Musty
87	846	9.60	47.00	6.0	7.5	4.5	7.0	3.5	7.5	
88	.....	8.00	21.30	7.0	7.5	4.5	4.5	4.0	5.0	Nutty, lacks pepper
89	M.L.	13.90	43.70	5.0	9.0	5.5	8.0	8.0	8.0	Sl. excessive sharpness
90	848	13.90	39.80	6.0	6.5	7.0	7.0	7.0	6.0	Excessive sharpness, soapy
91	848	14.50	45.00	6.0	8.0	6.0	7.5	6.0	6.5	Excessive sharpness, sl. soapy
112	.....	7.25	13.30	5.5	6.5	4.5	5.0	5.5	4.0	Unclean, sl. bitter
113	839	8.00	18.00	3.0	4.5	5.0	7.0	7.0	7.0	
114	843	7.90	14.40	4.0	3.0	4.0	4.0	6.0	4.0	Yeasty, unnatural, sl. sour
115	848	7.70	16.00	5.0	5.5	5.5	5.5	7.0	5.5	Sl. sour, sl. unclean
116	.....	7.00	13.00	4.0	3.0	4.0	5.0	5.0	5.0	Sour
117	846	16.70	33.00	3.5	5.0	6.0	7.5	7.5	6.5	Sl. sour, sharp
118	100	9.20	14.70	5.0	4.5	4.0	4.5	5.0	3.0	Unclean, sour, unnatural
119	M.L.	13.40	23.40	4.0	4.0	6.0	8.0	7.5	8.0	
124	.....	10.00	20.80	4.0	6.0	3.0	4.0	4.0	3.0	Sour, cheddary, unnatural
125	M.L.	13.50	40.70	5.0	5.0	5.5	6.5	7.5	4.5	Cheddary, soapy
126	843	10.30	19.60	3.5	5.0	4.5	5.0	7.5	4.5	Cheddary, sl. bitter
127	848	10.80	26.60	6.0	5.0	4.5	7.0	5.5	6.5	Cheddary, unnatural

[a] Total volatile acidity expressed as ml. 0.1 *N* acid/200 g. cheese

The results for all trials are shown in table 3. The various trials with a single strain were not grouped in the table because in each case the three lots of cheese should be compared with the control lot of cheese for that group.

In general, the strains of *C. lipolytica* used gave cheese which had a higher total volatile acidity and flavor score at 12 wk. than did the control cheese. Strains M. L., 848, 846 and 839 improved the cheese the most, whereas strains 47, 57, 100 and 438 had little or no effect in improving the cheese. The erratic results of the three trials with strain 843 cannot be explained satisfactorily at this time. In general, the volatile acidity values correlate with the flavor score values quite closely, indicating that the lipolytic action of the culture was at least one of the most important factors in flavor production.

DISCUSSION

The strain variation found in these trials indicates that the use of *C. lipolytica* cultures in milk for blue cheese requires careful selection of strains and adjustment of the amount of culture to use for each strain and for the intensity of flavor desired in the cheese.

The data presented tend to show some correlation of total volatile acidity to flavor score but very little correlation of fat acidity to total volatile acidity and flavor score.

The addition of a culture of a proven strain of *C. lipolytica* to pasteurized homogenized milk for blue cheese on a commercial scale would be much easier and less expensive than the preparation and use of an enzyme from the organism, or the use of a commercially prepared enzyme from the organism.

SUMMARY AND CONCLUSIONS

1. The addition of cultures of the lipolytic organisms *Alcaligenes lipolyticus*, *Achromobacter lipolyticum* and *Pseudomonas fragi* to pasteurized homogenized milk for blue cheese manufacture did not improve appreciably the flavor of the resultant cheese and did not cause any appreciable increase in total volatile acidity.

2. The addition of a culture of *Candida lipolytica* to milk for cheese making improved the flavor score and increased the total volatile acidity of the cheese.

3. Eleven strains of *C. lipolytica* from various sources differed markedly in their ability to increase the total volatile acidity and to improve the flavor of blue cheese made from pasteurized homogenized milk.

4. The results presented indicate a relationship of the total volatile acidity to the flavor score of blue cheese made from pasteurized homogenized milk.

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THE USE OF A MOLD–ENZYME PREPARATION IN THE MANUFACTURE OF BLUE CHEESE FROM PASTEURIZED HOMOGENIZED MILK[1]

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The production of enzymes by molds and the changes produced by these enzymes in dairy products have been recognized for some time. As early as 1910, Dox (2) reported that Laxa had noted that butter inoculated with *Penicillium glaucum* developed considerable acidity. Currie (1) concluded that during the ripening of Roquefort cheese, considerable fat was hydrolyzed by the water-soluble lipase produced by *Penicillium roqueforti*, and the characteristic peppery flavor and burning effect of the cheese on the tongue and palate were considered due to the caproic, caprylic and capric acids and their readily hydrolyzable salts which had accumulated. He noted that *P. roqueforti* will grow in Czapek's medium when sucrose is replaced by pure butterfat, tributyrin, ethyl butyrate, glycerin, butyric acid, or ammonium butyrate as the source of carbon. Thus, the mold not only has the power to hydrolyze fats but is able to utilize their components, also.

In cheese, it is believed that the enzyme diffuses beyond the growth zone of the mold and hence free fatty acids accumulate. Kirsh (4) reported that the water-soluble lipase of *Penicillium oxalicum* is highly non-specific, as it brings about almost the same amount of hydrolysis of esters, triglycerides of low molecular weight and a variety of emulsified oils. Fouts (3) observed that growth of *P. roqueforti* in sterilized cream appreciably increased the acidity of the fat.

Naylor, Smith and Collins (5) obtained maximum esterase production by *P. roqueforti* on Czapek's medium in which the sodium nitrate was replaced by 0.1 *N* ammonium chloride and to which 0.50 ml. of ethyl butyrate was added per 1,000 ml.. the reaction being adjusted to pH 4.5.

Thibodeau and Macy (7) found that *P. roqueforti* does not grow readily in a medium with an oxidation-reduction potential of over 400 millivolts. The addition of 0.1 per cent agar to Czapek's solution reduced the oxidation-reduction potential below 400 millivolts; addition of peptone or milk further reduced it. Presence of sugar in a medium tended to reduce lipase production by *P. roqueforti*. Maximum lipase was produced in Czapek's medium without sugar, but with 3.0 g. peptone and 3.0 g. butterfat per liter. The optimum activity of this lipase was over the pH range from 5.3 to 7.5, when the substrate was a 3.0 per cent butter oil emulsion in the presence of an acetate buffer. The production of lipase varied widely from strain to strain and seemed to be at a maximum as soon as the cultures had attained the stage of full sporulation. Sodium chloride in concentrations existing in blue cheese did not retard the action of either the lipase or the protease of the mold. These investigators found that the enzymes of *P.*

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roqueforti, added to blue cheese in the form of mycelium, produced cheese of fine quality which was ready for market in 5 mo. as compared to 10 mo. for the control cheese, unhomogenized milk being used for manufacture of the cheese.

METHODS

The methods for the manufacture of the cheese, the determination of fat acidity, the determination of total volatile acidity and the scoring of the cheese are given in a previous paper (6).

The medium used for making the mold-enzyme preparation contained sodium nitrate, 2 g.; monopotassium phosphate, 1 g.; potassium chloride, 0.5 g.; magnesium sulfate, 0.5 g.; ferrous sulfate, 0.01 g.; peptone, 3 g.; butterfat, 3 g.; agar, 5 g.; and water, 1,000 ml. Due to difficulty in sterilization, the butterfat was sterilized separately and added to the semi-solid medium at the time of inoculation. For sterilization, the medium was dispensed in 1-qt. bottles which were only half filled so the butterfat could be emulsified by shaking.

The mold-enzyme preparation was made by inoculating this medium with mold spores, emulsifying the melted butterfat into the medium and placing it in previously sterilized 2,800-ml. Fernbach flasks to a depth not to exceed 1 in. The mold was allowed to grow for 7 days at room temperature (70–75° F.) with shaking at 2-day intervals to break up the surface felt.

RESULTS

The mold-enzyme preparation made with four different mold strains was added to four lots of pasteurized homogenized milk at the rate of 0.55 per cent and the milk was made into blue cheese at once. The total volatile acidities, fat acidities, scores and defect comments for these lots of cheese are presented in table 1.

The total volatile acidities and fat acidities were high for the cheese 4 wk. old. The fat hydrolysis in lots 48 and 51 was excessive at 4 wk., as indicated by the defect comments. At 12 wk. all four lots of cheese had considerable peppery flavor but were too rancid to score for flavor and defect, indicating that too much of the preparation had been used. This is substantiated by the exceedingly high total volatile acidities and fat acidities at 12 wk. Mold strains 4 and 17 appeared to cause more fat hydrolysis than the other strains used.

The preceding trial indicated the possibility for use of the special mold-enzyme preparation if the right mold strain were selected, and if the proper concentration of mold-enzyme preparation were used in the cheese. Mold-enzyme preparations were made with each of five strains of mold and each preparation was added to three vats of milk at the rates of 0.05, 0.10 and 0.25 per cent. Repeat trials were made with two of the mold strains used. The total volatile acidities, scores and defect comments for all the trials are given in table 2.

All lots of cheese made with added mold-enzyme preparation had higher flavor and defect scores at 12 wk. than did corresponding control lots of cheese made without added mold-enzyme preparation. Two of the seven lots of cheese made with the 0.25 per cent concentration of added mold-enzyme preparation were criticized for being excessively sharp. Strains 4, 12 and 6 appeared to effect

TABLE 1

The effect of the addition of 0.55% of mold-enzyme preparation to pasteurized milk

Lot no.	Mold strain no.	Total volatile acidity[a]		Fat acidity[b]		Score						Comments for cheese 4 wk. old
						Mold		Flavor		Defect		
		4 wk.	12 wk.	4 wk.	12 wk.	4 wk.	12 wk.	4 wk.	12 wk.	4 wk.	12 wk.	
48	4	48.10	107.00	45.13	98.55	4.0	5.0	6.5	.....[c]	7.0	.....[c]	Excessive fatty acids
49	12	25.20	38.90	26.90	56.48	5.0	6.5	5.5	.....	4.0	.....	Some ketone, sour, unclean
50	14	22.40	64.00	20.38	50.83	4.0	6.0	4.0	.....	4.0	.....	Yeasty, unclean, lacking
51	17	63.00	99.60	48.76	112.63	6.5	4.0	6.0	.....	5.5	.....	Some ketone, excessive rancidity

[a] Total volatile acidity expressed as ml. 0.1 N acid/200 g. cheese.
[b] Fat acidity expressed as ml. 0.1 N acid/10 g. fat.
[c] Hydrolysis of fat too extensive to permit accurate scoring at 12 wk.

TABLE 2

The effect of the addition of varying amounts of mold-enzyme preparation of five strains of mold to pasteurized milk.

Lot no.	Mold strain no.	Mold enzyme prep. used	Total volatile acidity[a]		Score						Defect comments for cheese 12 wk. old
					Mold		Flavor		Defect		
			4 wk.	12 wk.	4 wk.	12 wk.	4 wk.	12 wk.	4 wk.	12 wk.	
		(%)									
92	4	0.00	8.60	28.60	4.0	7.0	3.5	3.0	4.0	2.0	Sour, musty, cheddary
93	4	0.05	9.30	22.30	4.5	7.5	3.0	5.5	7.0	7.0	Sl. nutty
94	4	0.10	9.80	26.00	3.5	4.5	4.5	6.0	6.0	5.0	Sour, sl. unnatural
95	4	0.25	9.50	36.50	3.5	4.5	5.5	7.0	4.5	7.0	Sl. nutty
96	12	0.00	10.00	15.20	6.0	9.0	3.0	4.0	5.5	3.5	Sour, fermented
97	12	0.05	7.50	15.70	8.0	7.5	3.5	6.0	2.0	5.0	Sl. sour, sl. fermented
98	12	0.10	7.50	28.40	8.0	8.0	4.0	6.0	3.0	4.0	Unclean, sour, fermented
99	12	0.25	12.00	32.80	8.0	6.0	5.5	7.5	4.0	7.0	Sl. fermented
100	6	0.00	6.00	22.50	5.0	8.0	4.0	2.5	6.0	2.0	Musty, unnatural
101	6	0.05	12.10	42.50	6.0	7.5	6.5	7.0	7.5	6.5	Unnatural
102	6	0.10	21.00	51.05	4.0	3.5	7.0	7.0	4.5	6.5	Unnatural
103	6	0.25	28.20	78.80	3.5	4.5	7.5	6.5	7.0	5.0	Excessive sharpness
104	13	0.00	9.10	14.50	3.5	5.0	3.0	3.0	2.0	2.0	Musty, sour, unnatural
105	13	0.05	9.70	14.50	7.5	5.0	4.5	5.0	4.5	5.5	Unnatural
106	13	0.10	9.25	15.60	6.5	4.0	4.5	5.0	5.5	6.0	Sl. unnatural
107	13	0.25	8.00	16.40	5.0	4.0	5.5	5.5	7.0	5.0	Unnatural
108	M_e	0.00	7.10	15.40	4.5	4.0	4.5	4.0	7.5	3.0	Musty, yeasty, sour
109	M_e	0.05	7.30	17.50	6.0	6.5	5.5	5.0	6.5	5.0	Unnatural, sl. sour, cheddary
110	M_e	0.10	11.30	17.00	3.5	2.5	5.0	5.5	4.0	5.0	Unnatural, sl. sour, cheddary
111	M_e	0.25	15.70	29.20	5.5	5.0	6.5	6.0	7.5	5.0	Unnatural, sl. sour, cheddary
140	6	0.00	11.80	17.80	5.5	5.0	4.0	2.5	4.5	2.5	Yeasty, sour, unclean
141	6	0.05	17.40	37.00	6.5	4.5	7.0	7.0	7.0	5.0	Sl. unclean, sl. fermented
142	6	0.10	29.00	57.00	4.5	4.0	7.5	7.5	7.0	7.5	Sl. soapy
143	6	0.25	43.05	80.50	4.0	4.5	6.5	6.5	5.0	6.0	Soapy, excessively sharp
144	M_e	0.00	8.05	20.50	7.5	5.5	3.0	3.5	4.0	3.0	Fermented, sour, sl. musty
145	M_e	0.05	10.60	29.00	7.5	5.0	4.5	4.5	4.5	4.0	Sour, sl. fermented
146	M_e	0.10	12.70	28.20	4.5	3.5	5.5	6.0	6.5	5.5	Sl. sour
147	M_e	0.25	25.00	50.70	3.0	4.5	6.5	7.0	6.5	6.5	Sl. soapy

[a] Total volatile acidity expressed as ml. 0.1 *N* acid/200 g. cheese.

the most improvement in flavor of the cheese, while strains 13 and M_6 effected the least improvement of the flavor. Strain 6 consistently gave the greatest increase in total volatile acidity of any of those used. It also gave the best flavor, particularly when the total volatile acidity was in the range of 30 to 60 ml. of 0.1*N* acid per 200 g. cheese.

Reduction of mold scores was observed in many instances when higher levels of mold-enzyme preparation were used, probably because of the known inhibitory effect of free fatty acids upon the development of microorganisms.

DISCUSSION

The use of a mold-enzyme preparation is a method for improving the flavor of blue cheese made from pasteurized homogenized milk. The preparation of the mold-enzyme material and the control necessary for its successful use would be somewhat more involved than the use of an enzyme or a culture in the milk for blue cheese manufacture on a commercial scale. The optimum amount of preparation to use appears to be about 0.25 per cent, which would be 25 lb. per 10,000 lb. vat of cheese. The preparation and incubation of that much material over a 7-day period would involve considerable labor, space and equipment. The mold-enzyme preparation also would serve as the source of mold inoculation of the cheese and, thus, would eliminate the cost of that item.

The reduction of mold growth frequently observed when the effective quantities of the mold-enzyme preparation were used would be undesirable commercially because of the desire of the consumer to purchase a cheese showing good mold growth. No suitable way to overcome this shortcoming has been found.

To be used effectively, the strain of mold would need to be chosen carefully and the amount of preparation to add to the milk would need to be determined for each mold strain under the particular manufacturing and marketing conditions of the individual manufacturing plant.

SUMMARY AND CONCLUSIONS

1. The proper addition of a mold-enzyme preparation to pasteurized homogenized milk increased the total volatile acidity and fat acidity and improved the flavor of blue cheese made from that milk.

2. The use of 0.55 per cent of the preparation in milk caused excessive fat hydrolysis in the cheese in all trials at 12 wk. The optimum amount to use appeared to be about 0.25 per cent for the conditions of these trials.

3. The strains of mold used varied greatly in the amount of hydrolysis which their respective mold-enzyme preparations would cause in cheese.

4. The effective use of this preparation in the improvement of blue cheese made from pasteurized homogenized milk will depend upon careful selection of mold strains to be used and proper adjustment of the amount of preparation to be used in the milk.

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THE RELATION OF CHEMICAL ANALYSES TO THE FLAVOR SCORES OF BLUE CHEESE MADE FROM PASTEURIZED HOMOGENIZED MILK[1]

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The work of Currie (3) has indicated that the characteristic peppery flavor of blue cheese and the burning effect on the tongue and palate are due to the caproic, caprylic and capric acids and their readily hydrolyzable salts. These accumulate in the cheese as the result of fat hydrolysis by the enzymes of the mold *Penicillium roqueforti*. Hammer and Bryant (5) succeeded in isolating methyl-n-amyl ketone from milk to which n-caprylic acid and mold spores had been added; they believed this compound was responsible for part of the characteristic flavor of blue cheese. Stokoe (14) and Davies (4) have explained the formation of methyl ketones by molds as the second step in an abnormal oxidation of fatty acids. Caprylic acid is the only fatty acid which would yield methyl-n-amyl ketone by this oxidation.

Lane and Hammer (9) found that blue cheese made from pasteurized homogenized milk showed more rapid development of volatile acidity, higher acid values on the fat and more typical flavor than cheese made from raw milk not homogenized; however, it did not ripen as fast as cheese made from raw homogenized milk. In cheese made from raw milk, the volatile acidity increased with increase in the time of ripening and older cheese contained a greater percentage of acids such as caproic, caprylic and capric. The acid values obtained on the cheese fat also increased with extended ripening. These investigators concluded that there was a general relationship between volatile acidities, fat acidities and flavor scores. They also found that the amount of soluble nitrogen in the serum from cheese increased with the age of the cheese but was not related to flavor. The amino nitrogen values and the nitrogen fractions soluble in trichloroacetic acid, ethyl alcohol, and phosphotungstic acid followed about the same pattern as the soluble nitrogen.

METHODS

The cheese was made and scored by the methods given in a previous paper (10). Volatile acidity of the cheese was determined by the method of Lane and Hammer (9). Fat acidity was determined by the method of Breazeale and Bird (2), after the fat was obtained and purified by the method of Lane and Hammer (9).

In making analyses for protein degradation, the cheese was made into a uniform suspension by a modification of the method of Knudsen and Sørensen (7). The cheese suspension was made by emulsifying 25 g. of cheese in 400 ml. of boiling 2.0 per cent sodium citrate solution by agitation in an Eskimo Whiz-mix (model 515JB) for 5 min. at high speed. The mixture was kept alkaline to

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brom thymol blue by adding alkali when necessary. This suspension was transferred quantitatively to a 500 ml. volumetric flask, cooled to 20° C. and made to volume with distilled water. Preliminary studies of this method of preparing the suspension, using completely dispersible peptone added to milk, indicated the citrate did not influence the nitrogen partition.

The nitrogen fractions soluble and insoluble in phosphotungstic acid were determined by the method of Lane and Hammer (8), with slight modification. Ten ml. of the cheese suspension (equivalent to 0.5 g. of cheese) was treated with a solution containing 50 ml. water, 15 ml. 25 per cent aqueous sulfuric acid and 5 ml. 20 per cent aqueous phosphotungstic acid for 16 to 18 hr. at room temperature. After filtration through a Whatman no. 12, 12.5 cm. fluted filter paper, the precipitate was washed three times with a solution of the same concentration as that used in the precipitation, and the total nitrogen was determined in the filtrate and in the precipitate by the Kjeldahl-Gunning-Arnold method of the Association of Official Agricultural Chemists (1). Copper sulfate was used as the catalyst and the mixed methyl red-methylene blue indicator of Johnson and Green (6) was used in the titration.

The nitrogen fractions soluble and insoluble in trichloroacetic acid were determined by a modification of the method of Lane and Hammer (8) in which cheese suspension equivalent to 0.5 g. of cheese was treated with a solution of 45 ml. water, and 5 ml. 20 per cent aqueous trichloroacetic acid for 16 to 18 hr. at room temperature. Further operations were the same as for the phosphotungstic acid procedure.

The amino nitrogen values for the cheese were determined by placing an amount of the cheese suspension equivalent to 1.0 g. of cheese in a 25 ml. volumetric flask, adding seven drops of glacial acetic acid to precipitate the casein, making to volume, filtering and making a Van Slyke amino nitrogen determination (15) on the filtrate. The values are expressed as milligrams of amino nitrogen per gram of cheese.

The statistical analyses of the data consisted of the calculation according to Snedecor (13) of the equation for the line of linear regression by the method of least squares and of the correlation coefficient (r), and also estimation of the significance of the correlation coefficient from table 7.3 of Snedecor (13).

RESULTS

The relation of fat degradation to flavor score. To learn more of the part which the fatty acids of lower molecular weight contribute to the flavor of the cheese, two series of trials were made in which varying amounts of some of the fatty acids were added to lots of pasteurized homogenized milk which then were made into cheese. In all cases, the fatty acids were added to a 100 g. quantity of melted butterfat, which then was homogenized into 1 qt. of milk and added to the cheese milk before setting with rennet. The same amount of butterfat alone homogenized into milk was added to the control lots. The kinds and amounts of acids added to each lot of cheese, as well as the scores and analyses of each lot of cheese, are presented in table 1.

TABLE 1

The effect of the addition of low molecular weight fatty acids on the score, volatile acidity and fat acidity of pasteurized milk blue cheese

Lot no.	Acid added to 100 g. butterfat[a]					Volatile acidity[b]		Fat acidity[c]	
	Butyric	Caproic	Caprylic	Capric	Lauric	4 wk.	12 wk.	4 wk.	12 wk.
	(*g.*)	(*g.*)	(*g.*)	(*g.*)	(*g.*)				
29	.....	.....	.....	.....	.....	8.00	18.40	4.26	20.00
30	.....	.....	2.0	.....	.....	7.45	15.50	4.18	8.45
31	.....	.....	10.0	.....	.....	15.30	18.90	5.25	14.72
52	.....	.....	.....	.....	.....	7.00	13.90	2.65	37.30
53	.....	0.4	0.2	0.4	.....	7.32	12.70	4.00	29.18
54	.....	0.4	0.2	0.4	0.8	6.40	20.60	3.17	29.98
55	0.6	0.4	0.2	0.4	0.8	6.00	14.20	2.74	18.27

Lot no.	Score						Defect comments for cheese 12 wk. old
	Mold		Flavor		Defect		
	4 wk.	12 wk.	4 wk.	12 wk.	4 wk.	12 wk.	
29	6.0	4.5	3.0	4.0	3.0	6.5	Flat, yeasty, nutty
30	5.0	5.5	6.0	6.0	7.0	7.0	Flat, nutty
31	4.0	3.5	6.0	4.5	5.0	4.0	Musty, caprylic acid
52	6.5	5.0	4.5	3.5	5.0	2.5	Nutty, unnatural
53	5.0	6.0	3.5	5.0	4.0	3.5	Sl. nutty, unnatural
54	4.5	7.5	4.5	7.0	5.5	5.5	Sl. nutty, unnatural
55	4.5	7.0	6.0	6.0	8.0	4.5	Sl. nutty, unnatural, unclean

[a] The 100 g. melted butterfat were homogenized into 1 qt. of milk and added to the 115 lb. of milk for cheese making.
[b] Volatile acidity expressed as ml. 0.1 *N* acid/200 g. cheese.
[c] Fat acidity as ml. 0.1 *N* acid/10 g. fat.

The cheese with added fatty acids showed definite improvement in flavor score over the control cheese; however, none was typical of good blue cheese, lacking the smoothness and fullness of flavor desired. The fat acidities and total volatile acidities increased considerably from the fourth to the twelfth week. The mold scores at 4 wk. were lowest in the cheese with the largest amounts of added fatty acids, although at 12 wk. there was a tendency for this relationship to be reversed. The fatty acids may be toxic to the mold until the mold is established and has started to utilize the acids in its growth. In the case of lot 31, so much caprylic acid was added that it probably was toxic during the entire 12 wk.

To determine whether a relationship between flavor score and fat acidity could be established with cheese made from pasteurized, homogenized milk, the fat acidities and flavor scores at 12 wk. for lots 29 to 31 and 52 to 55, as shown in table 1 of this paper, and lots 32 to 47 in table 1 of a previous paper (10) were analyzed statistically. The regression equation, degrees of freedom, correlation coefficient and significance of the correlation coefficient are shown in table 2. The data reveal no significant correlation between the fat acidities and the flavor scores of these lots of blue cheese.

The relationship of volatile acidity to flavor score for the lots of cheese to

TABLE 2

Statistical analysis of the relationship at 12 weeks of flavor scores of blue cheese to chemical analyses which indicate decomposition of fats and proteins

Analysis	Regression equation	d.f.[e]	r[f]	Significance of r
Fat acidity	$\hat{Y} = 1.05\,x + 11.15$	21	0.180	N.S.[g]
Volatile acidity[a]	$\hat{Y} = 4.61\,x + 3.01$	50	0.688	**[h]
Volatile acidity[b]	$\hat{Y} = 6.83\,x - 6.33$	29	0.507	**
Amino nitrogen	$\hat{Y} = .399\,x + 2.73$	29	0.518	**
Trichloroacetic N[c]	$\hat{Y} = .139\,x + 3.34$	29	0.358	*[i]
Phosphotungstic N[d]	$\hat{Y} = .144\,x + 1.76$	29	0.478	**

[a] *Candida lipolytica* added.
[b] Mold enzyme preparation added.
[c] Nitrogen fraction soluble in trichloroacetic acid.
[d] Nitrogen fraction soluble in phosphotungstic acid.
[e] Degrees of freedom.
[f] Correlation coefficient.
[g] Not significant.
[h] **Significant at the 1% level.
[i] *Significant at the 5% level.

which *Candida lipolytica* cultures were added (10) is shown in table 2. This relationship for these lots of cheese is highly significant on the basis of the correlation coefficient. The relationship of volatile acidity to flavor score for the lots of cheese to which a mold-enzyme preparation was added (11) is shown in table 2. The relationship is approximately the same as that for the cheese to which *Candida lipolytica* cultures were added.

The relation of protein degradation to flavor score. Amino nitrogen values and nitrogen fractions soluble and insoluble in phosphotungstic acid and in trichloroacetic acid were determined at 12 wk. for the cheese of lots 29 to 31 and 52 to 55 shown in table 1 of this paper and lots 32 to 47 and 56 to 63 shown in tables 1 and 2, respectively, of a previous paper (10). The relationship of amino nitrogen values, nitrogen fractions soluble in trichloroacetic acid and in phosphotungstic acid to flavor scores are shown in table 2. A highly significant correlation between the amino nitrogen values and the flavor scores of the cheese is indicated. The correlation between nitrogen fraction soluble in trichloroacetic acid and flavor score was significant at the 5 per cent level of probability. The correlation between nitrogen fraction soluble in phosphotungstic acid and flavor score is highly significant at the 1 per cent level of probability.

DISCUSSION

The data presented indicate no correlation of fat acidity to flavor score among the lots of cheese upon which fat acidities were determined. As the samples of fat were obtained with great difficulty, they possibly were not representative of all the fat in the cheese. The results in table 2 show a highly significant correlation of volatile acidity and flavor score for the lots of cheese made from pasteurized homogenized milk to which *C. lipolytica* cultures or mold enzyme prep-

arations had been added. In general, the cheese with highest flavor scores had volatile acidity values in the range of 30 to 55 ml. of 0.1 *N* acid per 200 g. of cheese. This range agrees well with that published by Peters and Nelson (12).

The data on protein degradation, although obtained on only a limited number of samples of cheese, indicate that protein degradation by the criteria employed is correlated with flavor development in blue cheese made from pasteurized, homogenized milk. This is contrary to the findings of Lane and Hammer (9) for cheese made from raw, homogenized milk. Possibly this is an associative rather than a direct relationship, since the flavor and aroma constituents which have been identified or suggested all have been derived from fat rather than from protein. The role which protein degradation plays in determining the desirable body characteristics of the cheese is considerable and contributes much to the consumer acceptance of the cheese. Protein degradation products undoubtedly combine with some of the fat degradation products or reduce the flavor intensity in other ways, with the result that some of the rawness of fat degradation products becomes integrated into a well balanced flavor, acceptable to the consumer. The present studies do not eliminate the possibility that cheese showing results of considerable proteolytic action but deficient in flavor could be produced under other experimental conditions.

The work with the addition of free fatty acids, while limited in scope, indicates definitely that these acids do contribute to the flavor of blue cheese. Their presence in excessive amounts, particularly when mold growth is restricted by their presence, results in cheese which has an undesirable rawness of flavor, presumably due to a disturbed balance in the mechanism of flavor production. The exact ratios of the various acids and the absolute amounts which should be used for optimum flavor production were not determined, since this portion of the study was designed only to obtain some direct evidence on the role of lower fatty acids in flavor development before study of microbial means of obtaining increased fat degradation in blue cheese was initiated. Under present rulings of regulatory officials, addition of the free fatty acids would not be permitted, even if the details of the procedure were to be worked out in such a way as to give a highly desirable type of flavor fortification to the cheese. Reliance on any such fortification also might prove extremely undesirable because flavor development might then precede proper body development, resulting in a product of reduced consumer acceptance.

SUMMARY AND CONCLUSIONS

1. The addition of low molecular weight fatty acids to pasteurized homogenized milk improved the flavor of blue cheese made from that milk. Such cheese lacked the fullness of flavor desired in typical blue cheese.

2. No correlation was found between fat acidities and flavor scores of blue cheese made from pasteurized homogenized milk.

3. A highly significant correlation was found between volatile acidity and flavor score of blue cheese made from pasteurized homogenized milk to which lipases or organisms producing lipases had been added. In general, the cheese

with the highest flavor scores had volatile acidities in the range of 30 to 55 ml. of 0.1 *N* acid per 200 g. of cheese.

4. The amino nitrogen values were correlated at a highly significant level with the flavor scores of blue cheese made from pasteurized homogenized milk.

5. Nitrogen fractions soluble in trichloroacetic acid and nitrogen fractions soluble in phosphotungstic acid were correlated significantly and highly significantly, respectively, to the flavor scores of blue cheese made from pasteurized homogenized milk.

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VITAL STUDIES ON BULL SEMEN USING TRIPHENYLTETRAZOLIUM CHLORIDE[1]

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Triphenyltetrazolium chloride (TTC) is colorless in solution but forms an insoluble red compound, triphenyl formazen, upon reduction (3). This property of TTC has lent itself to various physiological studies with living tissues involving reducing enzymes. This chemical has been used as a test reagent for seed germination studies (3), to demonstrate reducing enzyme systems in neoplasms and other living mammalian tissues (5), as a vital dye for stem tissues of plants (6) and for a wide variety of living tissues including bull sperm and the serum of bull sperm (3). The latter observation prompted these studies on the possible application of TTC in vital studies with bull semen and spermatozoa. Of particular importance would be its use as a stain to differentiate live and dead sperm (2) and as an indicator of sperm viability, much as the methylene blue reduction test is used (1).

MATERIALS AND METHODS

The semen used in these studies was collected with an artificial vagina from dairy bulls in the stud at the Dairy Research Farm, New Jersey Agricultural Experiment Station, Sussex. The semen diluter (4) used in toxicity trials was composed of equal part by volume of egg yolk and a 3.6 per cent solution of sodium citrate dihydrate, with sulfanilamide added at the rate of 3 mg. per ml. of complete diluter.

The duration of motility of spermatozoa incubated at 46.5° C. in a water bath was used as the measure of toxicity of the TTC. This duration of motility was expressed as a percentage of that of a control semen dilution containing no TTC.

To obtain sperm-free seminal plasma, the semen was centrifuged to throw down most of the spermatozoa and then the decanted plasma was filtered with a micro-Boerner centrifuge filter. This gives a seminal plasma that is absolutely free of sperm.

The amount of red color developed in any given trial with TTC was rated on a scale from 5 to 0, 5 being the highest and 0, no color. The best color development was an extremely brilliant red which started to develop within 5 min. after the addition of the TTC.

RESULTS AND DISCUSSION

To determine the toxicity of TTC, three separate semen samples were diluted with the egg yolk citrate-sulfonamide diluter at the rate of one part of semen to nine parts of diluter. TTC was added to 1 ml. portions of these diluted semen samples according to the schedule shown in table 1. The data for the three sam-

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TABLE 1

Toxicity of triphenyltetrazolium chloride

Dosage TTC/ml.	Av. duration of motility as % of controls	Av. color rating
(*mg.*)		
0 (Control)	100	0
0.001	94.1	0
0.01	86.8	0
0.1	75.0	1
1.0	33.8	2
5.0	8.8	3
10.0	2.8	2
20.0	<2.0	1

ples were averaged and the results are presented in the table. These data show that TTC is extremely toxic to bull spermatozoa. At the level of 5 mg. TTC per ml. the duration of motility of spermatozoa was 8.8 per cent of that of the controls. However, the red color development was greatest at this level. In subsequent experiments the level of 5 mg. of TTC per ml. of semen or diluter was used in rating color development or reduction of TTC.

That the reduction of TTC is proportional to the concentration of semen in the diluter is shown by the data in table 2. Several dilutions of a semen sample

TABLE 2

Concentration of semen in relation to reduction of TTC

Semen dilution	Dosage TTC	Color rating
	(*mg./ml.*)	
1 ml. semen	0	0
1 ml. semen	5	5
1 ml. 1:4 dilution of semen	5	4
1 ml. 1:9 " " "	5	3
1 ml. 1:99 " " "	5	0
1 ml. 1:999 " " "	5	0

were reacted with TTC and color reactions were rated at the end of 1 hr. incubation at 46.5° C.

Semen smears for microscopic examination were made from the tube with the highest color reaction; the spermatozoa were unstained or, at best, had an extremely light red stain.

The question arises as to whether the live spermatozoa, dead spermatozoa or seminal plasma may be responsible for the reduction of the TTC. In this connection several types of semen preparations have been reacted with TTC for 3 hr. at 46.5° C. at the rate of 5 mg. of TTC per ml. The data are presented in table 3. These trials seem to indicate that the reduction of TTC can not be caused by seminal plasma alone but rather by either live or recently killed spermatozoa. However, live spermatozoa gave a much more intense color reaction than did recently killed spermatozoa.

The high toxicity of TTC in semen, together with its extremely poor capacity

TABLE 3

Color reactions of various semen preparations

Sample no.	Description of sample	Color rating
1	1 ml. fresh semen	5
2	1 ml. plasma from fresh semen	0
3	1 ml. fresh semen—heat (46.5° C.) and cold (0° C.) shocked repeatedly to kill spermatozoa	2
4	1 ml. plasma from no. 3	0
5	1 ml. semen + 0.2 ml. toluene, incubated for 3 hr. to kill spermatozoa	2
6	1 ml. plasma from no. 5	0
7	1 ml. fresh semen heated to 82° C. for 20 min.	0

for staining spermatozoa, seem to preclude its use either as a vital stain for spermatozoa or as a general indicator of spermatozoa vitality.

SUMMARY

Triphenyltetrazolium chloride is readily reduced to triphenylformazen, an insoluble red compound, by fresh dairy bull semen. Semen in which the spermatozoa have been killed by heat- and cold-shocking or by treatment with toluene also has the ability to reduce the compound, but to a lesser degree. The heating of semen to 82° C. for 20 min. destroyed its reducing ability. Seminal plasma exhibited no ability to reduce the tetrazolium.

As judged by the effects of tetrazolium on the length of time which spermatozoa will maintain motility when incubated at 46.5° C., tetrazolium is very toxic. This together with its inability to stain spermatozoa adequately in its reduced state, precludes its use as a vital stain for spermatozoa or in measuring spermatozoa viability.

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THE INFLUENCE OF PASTURE AND EARLY RUMEN DEVELOPMENT ON THE CHANGES IN THE PLASMA CAROTENOIDS, VITAMIN A AND ASCORBIC ACID AND THE LIVER STORAGE OF CAROTENOIDS AND VITAMIN A OF YOUNG DAIRY CALVES

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The favorable results obtained from feeding relatively large amounts of good quality hay, coupled with rumen inoculations, as a means of initiating early rumen function and meeting some of the vitamin needs of young calves, suggested the feasibility of utilizing pasture, when available, in young calf feeding.

In an earlier communication (2), the changes in the blood plasma carotenoids, vitamin A and ascorbic acid during the first 6 wk. in calves fed milk and alfalfa hay with and without grain concentrates and with and without rumen inoculations with cud material from older cattle were presented. Calves fed on a ration consisting of whole milk and alfalfa hay had a much higher blood plasma carotenoid level than calves fed the same ration plus grain concentrates free choice. Little, if any, difference in plasma vitamin A was shown between the two groups. No effect from rumen inoculations could be detected in either the plasma carotenoids or vitamin A. However, a higher, more uniform level of ascorbic acid was maintained in inoculated calves fed only milk and alfalfa hay than in the uninoculated calves fed the same ration.

In accompanying reports (5, 6), the influence of the ration, including various proportions of grain concentrates to roughage ingestion, on the establishment of certain rumen microorganisms was shown.

This report presents the results obtained from feeding young calves on pasture with variations in supplemental hay and grain feeding and rumen inoculations. The changes in the blood plasma carotenoids, vitamin A and ascorbic acid, liver storage of carotenoids and vitamin A and the gain in body weight are included. The influence of pasture and rumen inoculations on the establishment of certain rumen microorganisms in the same calves is presented in an accompanying paper (7).

EXPERIMENTAL

Plasma carotenoids and vitamin A were determined by the procedure of Kimble (3), plasma ascorbic acid according to Mindlin and Butler (4) and liver carotenoids and vitamin A by an adaptation of the method of Guilbert and Hart (1).

Experiment with young calves. Fifteen calves of both the Jersey and Holstein breeds were assigned at birth to one of five groups. The calves in groups

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I and III were inoculated on the fifth, tenth, fifteenth and twenty-first days of age with cud material from older cattle (5) which were eating pasture. The calves in groups II, IV and V were not inoculated.

All the calves were allowed to nurse their dams for the first 3 days and then were fed whole milk at the rate of 0.9 lb. per 10 lb. of body weight, based on the birth weight. Beginning on the fourth day of age, the calves in the first four groups were tethered out during the day on a lawn type (bluegrass, white clover) pasture. The stakes were moved periodically so as to provide fresh grazing. Some variation in the quality of the pasture resulted from weather conditions, but for the most part, it was of good quality. The inoculated calves were kept separate from the uninoculated calves, both while on the pasture and when in the barn at night. One calf in each of the first four groups also was offered clover-timothy hay (second cutting) free choice while in the barn at night.

The three calves in group V were not given access to pasture. They were given clover-timothy hay (second-cutting) and a 14 per cent simple grain mixture free choice, beginning at 14 days. Groups I and II were not fed any grain supplement in addition to pasture and milk, while groups III and IV were given the 14 per cent simple grain mixture free choice, throughout the experiment. The experiment was terminated at the end of the sixth week.

Data showing the feed consumption and body weight gains are presented in table 1. The calves in all groups were bled as nearly as possible on the fourth and seventh days and weekly thereafter through the sixth week, and the samples were analyzed for carotenoids, vitamin A and ascorbic acid. Ten of the calves were slaughtered at 6 wk. of age and the livers were analyzed for carotenoids and vitamin A. The results of the blood and liver analyses for carotenoids are shown in table 2, those for vitamin A in table 3, and those for ascorbic acid in table 4.

Experiment with older calves. Blood plasma carotenoids, vitamin A and ascorbic acid analyses also were made periodically on five of the six older calves which are mentioned in the accompanying paper (7), after they were turned out to pasture. The five calves ranged in age from 62 to 96 days of age (average 71 days) on June 7, 1948, when they were put on a permanent bluegrass, white clover pasture.

Three of the calves had had their rumens inoculated artificially during the first 3 wk. after birth and two of them had not been inoculated. One of the latter two had picked up a partial inoculation in a natural manner. One of the three inoculated calves was given a fresh inoculation from a cow on pasture just before the calf was put on pasture. The changes in plasma carotenoids, vitamin A and ascorbic acid during the 5 wk. following the change from dry feeds to pasture are shown in table 5.

RESULTS AND DISCUSSION

Experiment with young calves. As shown in table 1, the calves that were offered hay ate a small amount in addition to the pasture. Most of the calves

TABLE 1

Feed consumption and body weight gains during the first 6 weeks of calves started on pasture

Group no.	Calf no.	Feed consumption: Whole milk[a] Age (days) 4–21	Whole milk[a] Age (days) 22–42	Hay[b] Age (days) 4–21	Hay[b] Age (days) 22–42	Grain[c] Age (days) 4–21	Grain[c] Age (days) 22–42	Pasture[d] First 6 wk.	Birth wt.	Body wt. gains at 21 d.	Body wt. gains at 42 d.	Remarks
		(*lb.*)	(*lb.*)	(*lb.*)	(*lb.*)	(*lb.*)	(*lb.*)	(*d.*)	(*lb.*)	(%)	(%)	
I	1H ♀	137	168	0	0	0	0	39	92	12.0	28.3	Inoculated. No grain fed.
	2J ♀	90	105	0	0	0	0	36	58	6.9	32.8	
	8H ♀	144	168	.....[e]	.....[e]	0	0	38	86	14.0	39.6	
	Av.	127	147					38	79	11.0	33.6	
II	3H ♂	162	189	0	0	0	0	39	101	14.9	42.5	Not inoculated. No grain fed.
	15J ♀	90	105	0	0	0	0	39	60	5.0	16.7	
	9H ♂	162	189	0.5	10.0	0	0	37	100	12.0	40.0	
	Av.	138	161					38	87	10.6	33.0	
III	4J ♀	90	105	0	0	1.0	9.5	39	55	16.4	40.0	Inoculated. Fed grain.
	5H ♂	162	158	0	0	5.0	8.5	35	109	19.3	47.7	
	11J ♀	82	105	6.0	2.5	5.0	5.5	39	48	4.2	37.5	
	Av.	111	123			3.7	7.8	38	71	13.3	41.7	
IV	6J ♂	90	105	0	0	1.0	7.0	39	62	16.1	40.3	Not inoculated. Fed grain.
	14H ♀	133	147	0	0	7.5	11.0	40	78	10.2	39.7	
	12J ♀	80	105	4.0	3.0	5.5	10.0	38	48	12.5	45.8	
	Av.	101	119			4.7	9.3	39	63	12.9	41.9	
V	7H ♂	144	168	1.0	9.0	1.0	9.0	0	87	13.8	58.6	Controls. No pasture. Fed grain and hay after 2 wk.
	10J ♂	86	92	2.0	3.5	1.5	3.5	0	46	2.2	22.7	
	13H ♂	118	168	0.5	4.0	0.5	6.0	0	93	5.4	26.9	
	Av.	116	143	1.2	5.5	1.0	6.2	0	75	7.1	36.1	

[a] Nursed dam first 3 d.
[b] Second cutting clover-timothy.
[c] 14% protein herd ration.
[d] Green lawn (Blue grass-white clover).
[e] *Ad libitum.* No record kept of wt.

TABLE 2

Changes in the blood plasma carotenoids during the first 6 weeks and total liver carotenoids at 6 weeks of age in calves started on pasture

Group no.	Calf no.	Age of animals (days)							Total liver carotenoids at 42 d.	Remarks
		4	7	14	21	28	35	42		
		(γ *carotenoids/100 ml. plasma*)							(γ)	
I	1H ♀	52.0	34.8	.....	35.6	.....	164.0	151.0	.....	Inoculated. No grain fed.
	2J ♀	.....	55.3	79.5	166.0	204.0	269.0	318.0	.....	
	8H ♀[b]	75.3	.....	65.8	37.3	38.8	134.0	186.0	.....	
	Av.				79.6		189.0	218.3		
II	3H ♂	34.9	.....	87.7	78.4	106.0	138.0	326.0	3,750	Not inoculated. No grain fed.
	15J ♀	41.2	.....	24.0	147.0	184.0	89.5[a]	169.0	3,021	
	9H ♂[b]	35.6	38.8	33.3	64.0	112.0	150.0	200.0	2,534	
	Av.	37.2		48.3	96.5	134.0	125.8	231.7	3,102	
III	4J ♀	117.0	.....	81.0	105.0	102.0	259.0	410.0	.....	Inoculated. Fed grain.
	5H ♂	42.0	.....	41.2	29.4	112.0	191.0	287.0	3,924	
	11J ♀[b]	.....	38.8	19.5	26.3	116.0	221.0	227.0	3,594	
	Av.			47.3	53.6	110.0	223.7	308.0	3,759	
IV	6J ♂	129.0	115.0	93.5	83.0	134.0	334.0	199.0[a]	3,273	Not inoculated. Fed grain.
	14H ♀	34.0	.....	34.9	27.9	26.3	83.0	193.0	2,007	
	12J ♀[b]	.....	23.2	9.9	75.5	244.0	363.0	392.0	.....	
	Av.			46.1	62.1	134.8	260.0	261.0	2,640	
V	7H ♂	20.2	25.4	28.6	36.4	25.6	27.9	29.5	497	Controls. No pasture. Fed grain and hay after 2 wk.
	10J ♂	47.7	.....	24.6	9.9	7.1	11.4	17.3	276	
	13H ♂	.....	15.8	9.9	12.9	23.2	16.5	15.8	507	
	Av.			21.0	19.7	18.6	18.6	20.9	427	

[a] Diarrhea when bled.

[b] Fed supplemental hay in the barn at night (Clover-timothy, second cutting).

TABLE 3

Changes in the blood plasma vitamin A during the first 6 weeks and total liver vitamin A at[b] 6 weeks of age in calves started on pasture

Group no.	Calf no.	Age of animals (days)							Total liver vitamin A at 42 d.	Remarks
		4	7	14	21	28	35	42		
		(γ *vitamin A/100 ml. plasma*)							(γ)	
I	1H ♀	12.0	14.2	.....	14.8	.....	14.9	13.5	.....	Inoculated. No grain fed.
	2J ♀	.....	20.4	18.9	14.8	15.7	17.5	9.9	.....	
	8H ♀[b]	23.8	.....	16.7	9.8	10.3	8.0	8.9	.....	
	Av.				13.1		13.5	10.8		
II	3H ♂	18.7	.....	10.6	17.4	14.6	12.0	11.7	11,637	Not inoculated. No grain fed.
	15J ♀	26.2	.....	21.7	18.4	16.5	13.3[a]	14.6	13,041	
	9H ♂[b]	14.9	15.4	14.3	9.8	9.6	9.4	20.4	10,784	
	Av.	19.9		15.5	15.2	13.6	11.6	15.6	11,821	
III	4J ♀	24.4	.....	15.4	10.8	9.2	8.9	14.9	.....	Inoculated. Fed grain.
	5H ♂	24.0	.....	13.1	12.8	13.7	13.2	15.1	19,488	
	11J ♀[b]	.....	26.2	13.7	10.5	10.4	13.2	13.9	8,524	
	Av.			14.1	11.4	11.1	11.8	14.6	14,006	
IV	6J ♂	26.6	19.7	12.6	13.3	14.9	15.4	12.7[a]	8,801	Not inoculated. Fed grain.
	14H ♀	17.2	.....	18.7	10.3	9.7	11.5	13.1	13,301	
	12J ♀[b]	.....	12.8	15.8	14.5	18.7	15.3	15.2	.....	
	Av.			15.7	12.7	14.4	14.1	13.7	11,051	
V	7H ♂	17.5	20.4	16.1	11.2	12.6	10.7	10.6	9,241	Controls. No pasture. Fed grain and hay after 2 wk.
	10J ♂	15.6	.....	12.0	7.3	3.1	4.5	4.8	2,448	
	13H ♂	.....	9.8	8.0	8.2	9.2	7.3	8.3	4,959	
	Av.			12.0	8.9	8.3	7.5	7.9	5,549	

[a] Diarrhea when bled.
[b] Fed supplemental hay in the barn at night (Clover-timothy, second cutting).

TABLE 4

Changes in the blood plasma ascorbic acid during the first 6 weeks in calves started on pasture

Group no.	Calf no.	Age of animals (days) 4	7	14	21	28	35	42	Remarks
		(*mg. ascorbic acid/100 ml. plasma*)							
I	1H ♀	0.40	0.33	.....	0.38	.....	0.31	0.39	Inoculated. No grain fed.
	2J ♀	.....	0.48	0.38	0.38	0.40	0.41	0.56	
	8H ♀[b]	0.55	.....	0.52	0.25	0.62	0.29	0.50	
	Av.				0.34		0.34	0.48	
II	3H ♂	0.45	.....	0.53	0.39	0.16	0.41	0.43	Not inoculated. No grain fed.
	15J ♀	0.59	.....	0.77	0.73	0.51	0.64[a]	0.50	
	9H ♂[b]	0.74	0.78	0.45	0.38	0.56	0.54	0.62	
	Av.	0.59		0.58	0.50	0.41	0.53	0.52	
III	4J ♀	0.67	.....	0.52	0.36	0.30	0.51	0.56	Inoculated. Fed grain.
	5H ♂	0.58	.....	0.36	0.29	0.45	0.54	0.44	
	11J ♀[b]	.....	0.82	0.36	0.49	0.93	0.64	0.42	
	Av.			0.41	0.38	0.56	0.56	0.47	
IV	6J ♂	0.69	0.47	0.59	0.34	0.59	0.51	0.39[a]	Not inoculated. Fed grain.
	14H ♀	0.60	.....	0.55	0.31	0.31	0.48	0.53	
	12J ♀[b]	.....	0.60	0.35	0.42	0.77	0.57	0.69	
	Av.			0.50	0.36	0.56	0.53	0.54	
V	7H ♂	0.90	0.41	0.30	0.20	0.54	0.54	0.42	Controls. No pasture. Fed grain and hay after 2 wk.
	10J ♂	0.41	.....	0.22	0.30	0.15	0.25	0.48	
	13H ♂	.....	0.39	0.25	0.60	0.81	0.19	0.42	
	Av.			0.25	0.37	0.50	0.33	0.44	

[a] Diarrhea when bled.
[b] Fed supplemental hay in the barn at night (Clover-timothy, second cutting).

TABLE 5

Changes in the blood plasma carotenoids, vitamin A, and ascorbic acid of calves given access to pasture at approximately 10 weeks of age

Calf no.	Before pasture[a]	Days after access to pasture[b]					Remarks (Before pasture)
		14	23	30	38	52	
			Plasma carotenoids (γ/100 ml.)				
16J ♂	42.8	368	416[c]	437	316	280	Inoculated
17J ♀	83.0	384	292[c]	399	398	305	Inoculated
18H ♂[b]	38.8	264	292	312	276	190	Inoculated
19H ♀	11.4	366	181	232	255	221	Partial natural inoculation
20H ♀	37.2	384	463	418	307	206	Not inoculated
Av.	42.6	353	329	360	310	240	
			Plasma vitamin A (γ/100 ml.)				
16J ♂	7.5	12.0	13.5[c]	12.2	15.0	20.8	Inoculated
17J ♀	8.2	21.1	21.6[c]	11.9	24.8	24.4	Inoculated
18H ♂[d]	8.2	11.7	13.9	10.8	9.9	14.8	Inoculated
19H ♀	12.8	21.4	21.2	22.0	22.3	19.4	Partial natural inoculation
20H ♀	6.9	16.6	20.2	16.4	15.5	17.0	Not inoculated
Av.	8.7	16.6	18.1	14.7	17.5	19.3	
			Plasma ascorbic acid (mg./100 ml.)				
16J ♂	.37	.55	.47[c]	.44	.41	.37	Inoculated
17J ♀	.60	.61	.23[c]	.35	.42	.46	Inoculated
18H ♂[d]	.64	.70	.54	.52	.55	.45	Inoculated
19H ♀	.32	.70	.48	.37	.42	.36	Partial natural inoculation
20H ♀	.68	.73	.78	.47	.47	.44	Not inoculated
Av.	.52	.66	.50	.43	.45	.42	

[a] Av. age 50 d.
[b] Av. age 71 d. at beginning of pasture June 7, 1948.
[c] Fed 1.5 lb. of grain daily after July 2, 1948.
[d] Freshly inoculated just before pasture period from cow eating pasture.

made satisfactory gains in body weight; however, considerable variation was observed. The calves fed on pasture and milk only (groups I and II) made an average increase in weight from birth to 6 wk. of 33.3 per cent while the calves that were fed grain in addition to pasture and milk (groups III and IV) averaged 41.8 per cent increase. No difference could be detected between the calves which were inoculated and those that were not, so far as their gains in body weight were concerned. This was true in both the grain-fed and no grain-fed groups.

Blood plasma carotenoids of the pasture calves (groups I, II, III and IV) increased rapidly reaching an extremely high but variable level(average 255 γ per 100 ml.) at 6 wk. of age. The calves fed grain concentrates (groups III and IV) increased at a somewhat slower rate during the first 4 wk., but during the fifth and sixth wk. increased much more rapidly than the no grain groups (I and II). Much of this apparent difference was due to the extremely high levels attained by two calves, 4J and 12J.

No difference was found between the inoculated and uninoculated calves so far as their plasma carotenoids were concerned, which is in agreement with the observations made previously (2) when hay was fed instead of pasture.

The control calves (group V) fed in the barn did not show any increase in plasma carotenoids during the entire 6-wk. period. As they were fed the same milk as the calves in the other groups, it is apparent that the increases in carotenoids in the other groups were due principally to utilization of carotenoids from the pasture grass.

Liver storage of carotenoids was approximately seven times higher, on the average, in groups I, II, III and IV than in group V. While the liver storage of carotenoids is somewhat variable, no marked differences among the first four groups can be seen. Unfortunately, liver storage data were not available in group I.

The changes in the plasma vitamin A, although quite variable indicated no marked difference among the pasture fed calves (groups I, II, III and IV). The plasma vitamin A levels in these calves were consistently much higher than those in the control group V. The vitamin A liver storage data do not show any clear cut difference among the pasture groups II, III and IV. No data were available in group I. The average liver storage of vitamin A of the pasture-fed groups was, however, more than twice that of the barn-fed calves.

The changes in the plasma ascorbic acid were variable, but were mostly in the normal range. No particular significance can be attached to the trends in the data. The calves in control group V at 14 days of age had an average level of 0.25 mg. per 100 ml. which is extremely low as compared to the other four groups.

Experiment with older calves. When the five older calves (average age 71 days) were turned out to pasture a marked increase in both plasma carotenoids and vitamin A occurred (table 5). Within 2 wk. the plasma carotenoids had increased more than eight times the pre-pasture level and the vitamin A nearly doubled. These high levels were maintained throughout the pasture period except for a decline in the carotenoids at the end, which probably was due to dry weather and maturing of the bluegrass. The accompanying rise in plasma vitamin A is another example of the increase in plasma vitamin A often observed concurrent with the fall from a high plasma carotene level to a lower level, as previously discussed (2).

No marked changes in the plasma ascorbic acid resulted from access to pasture, except that a temporary average increase was noted just after the change from dry feed to pasture.

SUMMARY AND CONCLUSIONS

An experiment was conducted to measure the influence of pasture and early rumen development on the performance of calves and as a means of meeting some of their vitamin needs. Twelve calves, one-half of which were rumen inoculated with cud material from older cattle, were tethered during the day on a lawn-type bluegrass-white clover pasture beginning at 4 days of age. One-half of the calves in both groups were fed a 14 per cent simple grain mixture free choice while on pasture. The pasture calves were compared to three calves fed in the barn on dry feeds. Milk feeding of all calves was limited to 0.9 lb. per 10 lb. of body weight at birth.

The calves on pasture were able to utilize the nutrients from the grass, as indicated by their high blood plasma and liver carotenoid and vitamin A levels plus satisfactory growth and appearance. The calves fed grain in addition to pasture increased in body weight more rapidly than those that did not receive grain. The plasma carotenoids of the pasture-fed calves averaged 255 γ per 100 ml. at 42 days of age. The average plasma ascorbic acid level at 14 days of age was also higher in the pasture-fed calves than in those that did not receive pasture. Otherwise, no marked differences were observed in the plasma ascorbic acid among the groups.

Rumen inoculations were not shown to affect the blood or liver vitamin levels which were observed, even though the inoculations and variations in the feed resulted in marked differences in the rumen microorganism picture (7).

Data also are presented showing the changes in plasma carotenoids, vitamin A and ascorbic acid before and after turning five older calves out to pasture. These calves had been fed in the barn, three with and two without rumen inoculation, prior to the pasture period.

Based on these findings, plus those presented in an accompanying paper (7), it is concluded that good pasture grass, when available, can be utilized by calves, even at an early age, as an effective means of meeting some of their vitamin needs and as a source of other nutrients.

The authors wish to acknowledge the assistance of John Tate, Miss Barbara L. Carson and C. E. Knoop in conducting this investigation.

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THE INFLUENCE OF PASTURE AND RUMEN INOCULATION ON THE ESTABLISHMENT OF CERTAIN MICROORGANISMS IN THE RUMEN OF YOUNG DAIRY CALVES

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The use of certain rumen microorganisms as indicators of the presence or absence of characteristic flora in young calves was described in a previous report concerning investigations of rumen development in these animals (4). The particular bacteria chosen for this purpose were observed quite regularly in samples from mature stock consuming usual mixed rations. Generally, they were present, especially in samples from calf rumens, in sufficiently limited numbers to permit detecting variations in concentrations. Furthermore, they were among the rumen bacteria which can be identified most readily in stained smears in so far as morphology and staining characteristics permit.

In the previous report (4), four of these microorganisms were referred to as being associated with a high proportion of hay ingestion. Large Gram-positive cocci in closely knit pairs were described, and these were referred to as the first hay-group bacteria. The following three bacteria made up the second hay-group and were described as (a) large Gram-positive, thick, fairly square-ended rods, (b) very large Gram-negative, cigar-shaped rods and (c) smaller Gram-negative, short rods in fours and multiples of four in shapes suggestive of window panes. Medium sized, comparatively thin, Gram-positive rods, which sometimes stained in a granular manner, were among the bacteria observed to be associated with the consumption of high proportions of grain.

The bacteria which were observed to be associated with a high proportion of hay ingestion and also the protozoa sometimes failed to become established in young calves. Segregation of the calves from the older stock which apparently cut off their source of inoculum for some of these microorganisms, and the failure of the calves to ingest combinations of feeds suitable for development of the microorganisms were the two reasons involved. Under some conditions, the lack of usual microorganisms in the rumen appeared to be associated with a lowered state of health in these young animals (2, 4, 5). The feeds involved in the above investigations were dry-feed rations consisting of hay and grain. The present report concerns results of a study of the rumen microorganisms in calves on pasture, both with and without hay and grain supplements.

EXPERIMENTAL

Two groups of Holstein and Jersey calves were used. The first consisted of 12 calves which were tethered each day on lawn pasture beginning at 4 days of age. At night, they were kept in the barn in separate groups. They received per day 0.9 lb. of milk for each 10 lb. of body weight at birth. Half of them received

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rumen inoculations in the manner described previously (4) when they were 5, 10, 15 and 21 days old, using cud materials from cows on pasture. Some of the calves were fed either mixed hay or a 14 per cent protein grain mixture, or both, free choice while they were in the barn at night.

The second group was composed of six calves which were between 39 and 96 days of age, and which averaged 66 days at the time they were given access to pasture during the day beginning on June 7. They had been receiving milk, grain, and mixed hay. These calves received either whole milk or separated milk during the pasture feeding period. They were kept in the barn at night and were turned out each day on a mixed bluegrass and clover pasture in which growth of coarse types of weeds was fairly heavy in some places. Two of these calves received, in addition, 1.5 lb. per day of the grain mixture beginning on July 2. Four had received rumen inoculations during their first 3 wk. of age, and two of these four were given reinoculations with cud materials from cows on pasture. The rumen of the fifth had become partially inoculated in a natural manner, and the sixth was an uninoculated animal which had been raised in comparative segregation.

Rumen samples were collected repeatedly from both groups and examined in the manner described previously (4). Protozoa were examined in the fresh state and Gram-stained smears were relied on for bacterial observations. The presence or absence of the same bacteria as formerly described was determined and the same designations that had been assigned to these have been followed. An attempt was made to grade the samples according to the relative numbers of these particular microorganisms which were observed; this consisted of making rough estimates of the concentration and assigning values ranging between 0 and 4.

RESULTS

Experiment with young calves. The results of the examinations made on rumen samples collected from the 12 younger calves when they were 3 and 6 wk. old are presented in table 1. The treatment as regards inoculation, the type of feed given each calf, and the ratings assigned to the concentrations of some of the microorganisms in the rumen samples are included.

Protozoa were present in the samples from all six of the inoculated calves at 3 wk. of age and were present in great numbers at 6 wk., but were completely absent in the samples obtained from the uninoculated calves. Of the bacteria which had been observed to associate with hay ingestion (4), those in the first hay-flora group were present in samples from all but one of the inoculated calves at 3 wk. of age and in all at 6 wk. They were present in samples from two of the six uninoculated calves at 3 wk. and in those from four of them at 6 wk. of age. Bacteria designated as belonging to the second hay-flora group were present in samples from only one of the six inoculated calves at 3 wk. and in but four at 6 wk. of age. The two calves in whose rumen samples they were absent were receiving grain free choice. These bacteria were never observed in samples from the six uninoculated calves. Within their respective groups, samples from the

TABLE 1

Ratings indicating the relative concentration of certain microorganisms in rumen samples from young calves following access to pasture beginning at 4 days of age

Calf	Feed	Rumen microorganisms ratings[a] at:					
		3 wk. of age			6 wk. of age		
		Protozoa	Hay-flora		Protozoa	Hay-flora	
			I	II		I	II
	Inoculated						
1 H	Pasture alone	3	2	0	3	3	2
2 J	Pasture alone	2	3	1	3	3	2
8 H	Plus hay	2	3	0	3	4	3
	Uninoculated						
3 H	Pasture alone	0	0	0	0	1	0
15J	Pasture alone	0	0	0	0	0	0
9 H	Plus hay	0	2	0	0	2	0
	Inoculated						
4 J	Plus grain	2	1	0	3	1	0
5 H	Plus grain	1	0	0	3	1	0
11J	Plus hay and grain	3	2	0	3	3	1
	Uninoculated						
6 J	Plus grain	0	1	0	0	1	0
14H	Plus grain	0	0	0	0	1	0
12J	Plus hay and grain	0	0	0	0	0	0

[a] Ratings: 1 = few; 2 = moderate numbers; 3 = many; 4 = masses.

two calves receiving hay in addition to the pasture were rated as having the higher concentrations of the bacteria noted to associate with hay ingestion.

Although not shown in table 1, samples from all 12 calves contained varying numbers of the Gram-positive rods designated in the previous report (4) as associated with grain ingestion. These were especially prevalent in samples from calves consuming a high proportion of grain during the first 4 wk.

The general appearance and growth of all the calves was comparatively good. Their gains in weight are reported in an accompanying paper (3). Four calves suffered from attacks of diarrhea, which were of short duration and not severe, and all recovered without treatment. Two of these calves (numbers 5 and 11) had received rumen inoculations and two (numbers 12 and 15) had not; one received pasture alone (number 15), one pasture plus grain (number 5) and the other two pasture plus both grain and hay (numbers 11 and 12).

Three other uninoculated calves which were born during the same period were kept in the barn continuously, were given milk and fed similar hay and grain free choice starting at 14 days of age. The results of examinations of rumen samples were rather similar to those previously obtained for calves handled in this manner (4). Protozoa were missing from all samples, and bacteria of the hay-flora groups were practically absent from all samples. On the other hand, great numbers of the Gram-positive rods observed to associate with a high proportion of grain ingestion were present in all samples. Of these three barn-fed

calves, two suffered from mild attacks of diarrhea of short duration, which desisted without treatment.

Experiment with older calves. Data collected on rumen samples obtained from the older group of six calves on several representative days are presented in table 2. The ratings assigned on the basis of the relative concentration in the rumen samples of the particular microorganisms which were being observed are given. No marked change in the relative numbers of protozoa were noted throughout the period of observation. The two groups of organisms which have been observed to associate with hay ingestion tended to decrease in the samples during the first few days the calves were on pasture, but soon regained their former status. During this period, small Gram-negative short rods were especially prominent in all the samples. When the calves were first turned out, the pasture was particularly lush. Besides these changes in the microflora, the plasma carotenoids also tended to vary considerably (3), indicating that the character of the pasture was more than likely involved in both variations. The calf having a partial inoculation, which was acquired in a natural manner, developed a rumen flora and fauna which appeared quite comparable to those of the inoculated animals after associating with them for approximately 2 wk. Microorganisms in rumen samples from the uninoculated animal failed to become similar to the others, even though they progressed somewhat in this direction. However, characteristic microorganisms readily were established when the animal received an inoculation with cud materials from an older animal on July 30. A rumen sample obtained on August 23 was rated for protozoa as 3, for hay-flora group I as 3, and for hay-flora group II as 2. It was noted that the same rather large Gram-positive rods frequently seen in other uninoculated calves were present in many of the samples obtained from this calf.

The percentage increases in weight on August 2 over the weights on June 2 were 61.0 and 75.5 per cent for the two inoculated calves which received grain part of the time. For those which did not receive grain, they were 53.5 and 62.6 per cent for the inoculated calves, 58.1 per cent for the naturally inoculated calf and 51.7 per cent for the uninoculated animal. The inoculated calf which gained 53.5 per cent was a twin of the uninoculated calf. The latter calf suffered from recurrent mild diarrhea until after it received the rumen inoculation on July 30. Its tail and hind legs were fouled with feces almost continuously, which was seldom observed in any of the other calves. It also had a noticeably rougher hair coat.

DISCUSSION

The observation that the same rumen microorganisms were established as readily in the rumens of calves eating pasture as when they were consuming dry feeds is in line with the findings of others, including Bortree *et al.* (1), that these feeds promote the development of rather similar rumen flora.

The absence in the uninoculated calves of certain characteristic microorganisms which were present in the rumens of inoculated calves indicates that calves having access to pasture are in no better position than those being raised on dry feeds as regards obtaining these microorganisms from sources other than the

TABLE 2

Ratings indicating the relative concentration of certain microorganisms in rumen samples from calves given access to pasture at approximately 2 mo.

Calf	Rumen microorganism ratings[a] on:														
	June 5			June 9			June 23			June 30			Aug. 2		
	Protozoa	Hay	Flora	Protozoa	Hay	Flora	Protozoa	Hay	Flora	Protozoa	Hay	Flora	Protozoa	Hay	Flora
		I	II		I	II		I	II		I	II		I	II
16 J[b]	3	1	0	3	0	1	3	0	2	3	3	3	3	3	3
17 J[b]	4	1	1	3	0	0	3	1	3	3	3	3	3	2	1
18 H[b]	2	3	1	3	2	0	3	1	2	3	3	3	3	2	2
21 J[b]	3	3	2	3	2	1	3	1	1	3	3	2	3	3	2
19 H[c]	2	1	0	2	0	0	3	1	1	2	2	0	3	2	1
20 H[d]	0	2	0	0	3	0	1	0	0	1	1	0	2	2	0

[a] Ratings of microorganisms present: 1 = few; 2 = moderate numbers; 3 = many; 4 = masses.
[b] Rumen inoculated when 3 wk. old (18H and 21J reinoculated June 7).
[c] Partial natural inoculation.
[d] Uninoculated.

bovine rumen. In the case of the younger calves which were eating lawn pasture, the lack of characteristic rumen microorganisms did not appear to be of much consequence in so far as gain in weight (3) or general health was concerned.

The slowness with which the uninoculated calf in the older group developed rumen flora and fauna similar to the others while on pasture with inoculated calves was rather unexpected. The data collected on this single calf cannot, of course, provide more than a slight indication of what may occur in similar animals under such circumstances. However, it does suggest the possibility that failure of characteristic flora and fauna to become established in the rumens of young animals, which are forced to depend upon pasture utilization to meet their nutrient requirements, may limit their ability to efficiently utilize some types of pasture.

The comparatively normal existence which frequently is possible for calves, even though they lack some of the usual rumen microorganisms, probably is due to the fact that substitute organisms can do a creditable job. However, microorganisms that have developed over a long period of time in the environment of the rumen, would be expected to function most efficiently in this organ.

When the role that segregation can play in the control of the spread of some infectious disease organisms is considered, the effect of such a management procedure on the transfer of rumen microorganisms from animal to animal can be appreciated more readily.

SUMMARY

Rumen inoculations with cud materials from cows on pasture were given six of twelve calves which were fed milk and placed on lawn pasture at 4 days of age. Rumen protozoa and certain bacteria, used as indicators of the presence of varieties characteristically associated with a high proportion of hay ingestion, readily were established in all inoculated calves. The bacteria were established in a relatively less degree in two of the calves which received grain supplement free choice. Protozoa did not develop in the uninoculated calves. Some characteristic bacteria became established in four of the six uninoculated calves by 6 wk. of age, but were limited to one of the observed varieties and were relatively few in number.

Characteristic rumen microorganisms became established only in relatively limited numbers in a milk-fed, uninoculated, 2-month old calf after being in a pasture for 7 wk. with four rumen-inoculated calves of similar age. The marked difference in microorganisms was rectified following rumen inoculation. Prior to inoculation, this calf had recurrent mild diarrhea and a comparatively rough hair coat while on pasture, but its percentage gain in body weight was almost equivalent to an inoculated twin.

Characteristic rumen microorganisms can be established in young calves on pasture when they are inoculated with cud materials from older cattle and when grain is not fed in excessive amounts. Calves possibly may be limited somewhat in their ability to utilize certain pastures, if characteristic rumen microorganisms are lacking.

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PREPARTUM MILKING. III. THE PLASMA LEVELS OF CAROTENE AND VITAMIN A IN CALVES FROM DAMS MILKED PREPARTUM AND IN CALVES FROM DAMS MILKED POSTPARTUM[1]

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Prepartum milking results in a marked decrease in both the carotene and vitamin A content of colostrum (3). Since colostrum contributes a large proportion of these nutrients to the young calf, it is of value to know what effect prepartum milking of the dam has on these metabolites in the young calf. The purpose of this study was to determine the effect of prepartum milking of the dam on the carotene and vitamin A content of the plasma of the neonatal calf. Secondarily, these factors were studied in relation to two dietary regimes.

EXPERIMENTAL

Animals. A detailed description of the treatment, changes in certain blood constituents and composition of the colostrum of the dams of the calves used in this experiment has been reported previously (2, 3). Briefly, the dams were divided into four experimental groupings: 1-A, postpartum milked—basal ration; 1-B, postpartum milked—basal ration + 1 million USP units of vitamin A daily for 30 days prior to the calculated parturition date; 2-A, prepartum milked for 10 days prior to calculated parturition date—basal ration; and 2-B, prepartum milked—basal ration + vitamin A. There were nine calves from dams in group 1-A, ten calves from dams in group 1-B, and 11 calves each from dams in group 2-A and group 2-B.

The calves were not allowed to nurse but were removed immediately after birth to individual pens in a separate portion of the barn. There the Ayrshire and Holstein calves received 6 lb. daily of their dams' colostrum and milk for the first wk., and 7, 8, and 9 lb. of herd milk for the second, third, and fourth wk., respectively. Similar values for the Guernsey and Jersey calves were 5, 6, 7, and 8 lb. All colostrum and milk were fed twice daily in nipple pails. At the beginning of the second wk. of age, each calf had free access to water, commercial dry-calf starter and mixed grass and clover hay. Each calf was weighed at birth and at weekly intervals thereafter to 4 wk. of age. In cases of scours, the milk allowance was reduced to one-half; and in three calves it was necessary to inject intravenously, at the rate of 1 grain per lb. of body weight, a 25 per cent solution of sodium sulfamethazine as a therapeutic agent.

Samples and Analyses. Venous blood samples were drawn at birth and at weekly intervals thereafter to 4 wk. of age. The samples immediately were

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chilled to 4° C. and centrifuged, and plasma carotene and vitamin A were determined by the method of Kimble (7). Standard statistical procedures (5, 10) were used to test for differences between treatments. In the case of liveweight, the method of Wishart (14) was used, as well as methods outlined in Snedecor (10).

RESULTS

Data on the content of carotene and vitamin A in the blood plasma and liveweight at birth, and at weekly intervals to 4 wk. of age, are given in fig. 1 to 3. Prepartum milking resulted in lower levels of both plasma carotene and plasma vitamin A. Secondarily, the feeding of supplementary vitamin A to the dam prepartum resulted in higher levels of vitamin A in the plasma and in a depression in the levels of carotene in the plasma.

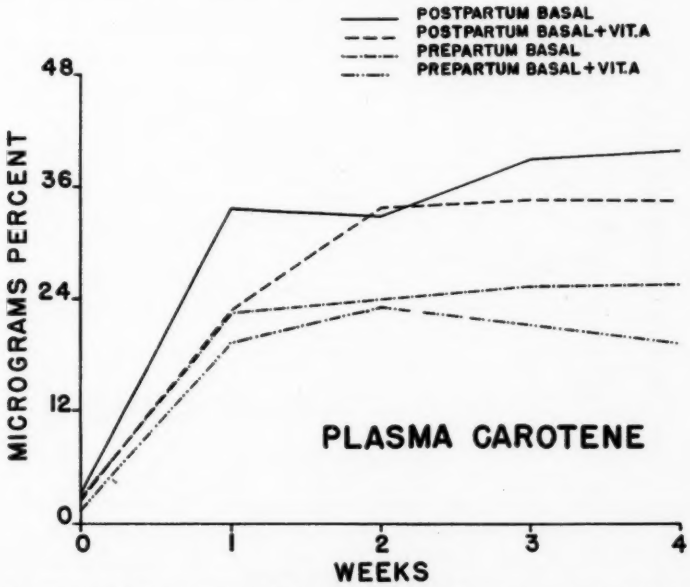

FIG. 1. The effect of prepartum milking of the dam on the carotene content of the plasma of the dairy calf.

Plasma carotene (fig. 1) was affected significantly by treatment. Calves from dams milked prepartum had lower average carotene plasma values ($P<0.01$) for the entire experimental period, and also lower average values for the period of 1 through 4 wk. of age ($P<0.05$), than those values for calves from dams milked postpartum only. A similar difference ($P<0.05$) existed at 1 and at 2 wk. of age. The feeding of supplementary vitamin A prepartum resulted in lower levels of plasma carotene, but these differences were not statistically significant.

Plasma vitamin A levels (fig. 2) were lower ($P<0.05$) from 1 wk. of age through 4 wk. in calves from dams milked prepartum. The feeding of supplementary vitamin A prepartum significantly ($P<0.01$) raised the blood plasma levels of vitamin A for the entire experimental period. Also, the blood plasma levels of vitamin A at birth were higher ($P<0.001$) in those calves from dams fed supplementary vitamin A than in calves from dams fed the basal ration alone.

The liveweight increases (fig. 3) were greater in those calves from dams milked only postpartum and those calves from dams receiving supplementary

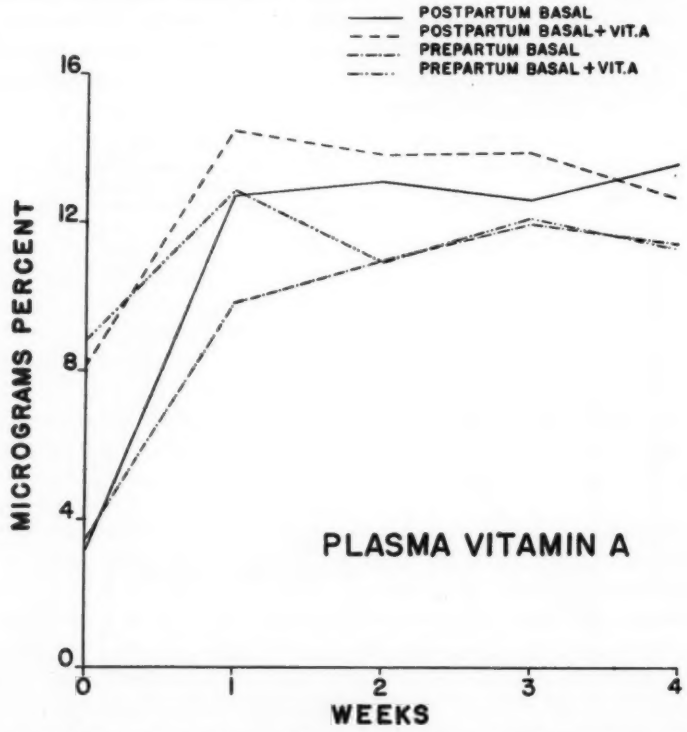

FIG. 2. The effect of prepartum milking of the dam on the vitamin A content of the plasma of the calf.

vitamin A. An analysis of the actual gains during the 28-day experimental period and their linear coefficients, or either of these measures adjusted to the birth weight of the individual calves, failed to reveal statistically significant differences between treatments.

Four calves from dams milked prepartum and fed the basal ration (group 2-A) had scours for a period of 1, 1, 3, and 8 days, respectively. Two of these received intravenous injections of 25 per cent sodium sulfamethazine. In group 2-B, two calves had scours for 1 day each. No scours were observed in calves from

dams milked postpartum and fed the basal ration (group 1-A). One calf in group 1-B had scours for a total of 7 days and received intravenous injections of 25 per cent sodium sulfamethazine. Conversion of the percent days free from scours to an angle corresponding to the percentage, and analysis of variance of the angles showed no statistical differences due to treatment.

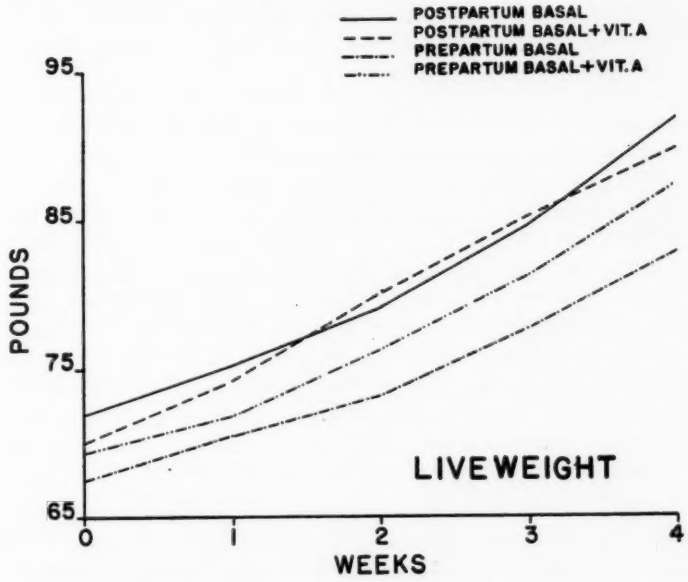

FIG. 3. The effect of prepartum milking of the dam on the liveweight changes of the dairy calf.

DISCUSSION

These data indicate that both management and ration during the prepartum period can influence the blood plasma levels of carotene and vitamin A in the young dairy calf.

Previous workers (6, 8, 13) have reported difficulty in raising calves from cows milked prepartum, and Keyes *et al.* (6) have indicated that oral administration of a carotene preparation would alleviate scours and general inactivity in calves from dams milked prepartum. In this study, the blood plasma levels of vitamin A of the calves from dams milked prepartum and receiving the basal ration alone were, on the average, slightly above 10γ per cent, which Boyer *et al.* (1) have indicated as adequate. There appears to be the possibility that a suboptimum intake of vitamin A might exist in calves when their dams receive limited amounts of carotene in their ration and are milked prepartum. However, other factors may influence the nutrition of the calf when its dam is prepartum-milked since not only is there a quantitative change in the contituents of colostrum, but

also a qualitative change as reviewed elsewhere (3). Although the vitamin A content of the colostrum of the dams milked prepartum and fed supplementary vitamin A was significantly greater than that for dams milked postpartum and fed no supplementary vitamin A (3), calves from dams in the former group did not maintain as high plasma vitamin A levels after 2 wk. of age as did calves from dams in the latter group. This suggests that colostrum contains a factor(s), apparently not found in milk, which results in greater efficiency in the utilization of vitamin A. Previous workers (4, 11) have indicated such, but more direct measurements are needed.

The increase in blood plasma levels of vitamin A in those calves from dams fed supplementary vitamin A during the prepartum period confirms work previously reported (12). In addition, the cases of scours, although few and not statistically significant, are in line with the previous report. The depression in carotene, likewise not statistically significant, is of interest; since intrauterine influences of supplementary vitamin A feeding apparently carry over into the neonatal calf under "normal" conditions of feeding and management.

The greater but not statistically significant liveweight gains in calves from dams fed supplementary vitamin A prepartum is of interest. Previous workers (9, 12) have demonstrated significantly greater liveweight gains in neonatal Holstein calves due to prepartum feeding of supplementary vitamin A, and in Holstein heifers fed supplementary vitamin A directly. The lower but not significant weight gains in those calves from dams milked prepartum well might be due in part to suboptimum intakes of vitamin A.

SUMMARY

The effect of prepartum milking of the dam, for 10 days prior to the calculated parturition date, on the plasma carotene and vitamin A levels, liveweight changes, and incidence of scours in 41 young dairy calves has been studied. Secondarily, the effect of feeding one million USP units of vitamin A daily for 30 days prepartum was measured.

The data indicate that prepartum milking significantly lowers the level of blood plasma carotene and vitamin A in calves from 1 wk. through 4 wk. of age, as compared to those values for calves from dams milked postpartum only. The feeding of supplementary vitamin A prepartum resulted in significantly greater blood plasma levels of vitamin A for the entire experimental period and lower but not statistically significant blood plasma carotene levels. The differences between treatments, in liveweight and incidence of scours, were not statistically significant.

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ASSOCIATION ANNOUNCEMENT

COLLEGIATE STUDENTS' INTERNATIONAL CONTEST IN JUDGING DAIRY PRODUCTS

Los Angeles, Cal.—Oct. 23, 1949

Teams from 18 State Agricultural Colleges, participated in this, the fifteenth annual Contest sponsored by the Dairy Industries Supply Association, Inc., and the American Dairy Science Association.

Following is a list of those who won high standings in the Contest:

ALL PRODUCTS

Individuals

1. Herbert Ruggles, Iowa State College
2. Richard Jackson Stanley, Mississippi State College
3. Russell J. Moe, University of Minnesota
4. Donald E. Miller, University of Illinois
5. John R. Tedford, University of Connecticut
6. Gene D. Lower, Ohio State University
7. Lee R. Morgan, Utah State Agricultural College
8. Harold A. Ramsey, Kansas State College
9. Sam Louis Swett, Mississippi State College
10. Robert K. Wight, Iowa State College

Teams

1. Mississippi State College
2. University of Connecticut
3. Iowa State College
4. Kansas State College
5. University of Minnesota
6. University of Massachusetts
Tie 7. Michigan State College
Tie 7. State College of Washington
9. Utah State Agricultural College
10. University of Illinois

BUTTER

Individuals

1.	Russell J. Moe, University of Minnesota	13.25
2.	Raymond G. Otto, University of Minnesota	14.67
3.	Herbert Ruggles, Iowa State College	14.74
4.	Donald E. Miller, University of Illinois	15.84
5.	Philip J. Blanchard, Jr., University of Massachusetts	15.92
6.	John R. Tedford, University of Connecticut	17.00
7.	Duane D. Walter, State College of Washington	17.50
8.	Warren C. Jones, Texas A & M College	18.17
9.	William R. Thomas, Oklahoma A & M College	18.33
10.	Edwin R. Frankel, Michigan State College	19.00

Teams

1.	University of Minnesota	57.92
2.	University of Massachusetts	60.93

3.	Michigan State College	63.51
4.	University of Connecticut	64.34
5.	State College of Washington	64.51
6.	Mississippi State College	68.10
7.	Kansas State College	68.52
8.	University of Illinois	68.68
9.	Oklahoma A & M College	71.50
10.	Agricultural & Mechanical College of Texas	72.34

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Individuals

1.	Sam L. Swett, Mississippi State College	26.08
2.	Richard Jackson Stanley, Mississippi State College	27.59
3.	Dee R. Morgan, Utah State Agricultural College	30.75
4.	John R. Tedford, University of Connecticut	31.02
5.	Dee McDonald Graham, Mississippi State College	31.17
6.	Max R. Hogan, Utah State Agricultural College	31.26
7.	Marvin Eskin, Michigan State College	31.92
8.	James D. Yoder, University of Nebraska	32.51
9.	Alfred Cohn, Michigan State College	32.84
10.	William Edmondson, University of Connecticut	32.93

Teams

1.	Mississippi State College	84.84
2.	Utah State Agricultural College	100.60
3.	Michigan State College	102.00
4.	Iowa State College	104.60
5.	University of Nebraska	105.18
6.	University of Connecticut	106.38
7.	University of Massachusetts	110.43
8.	Kansas State College	110.61
9.	University of Minnesota	111.86
10.	Texas Technological College	112.51

ICE CREAM

Individuals

1.	Roger W. Hunt, University of Connecticut	22.67
2.	Herbert Ruggles, Iowa State College	25.00
3.	Harold A. Ramsey, Kansas State College	25.84
4.	Donald E. Miller, University of Illinois	27.34
5.	Richard Jackson Stanley, Mississippi State College	27.67
Tie 6.	Robert K. Wight, Iowa State College	28.00
Tie 6.	Philip J. Blanchard, Jr., University of Massachusetts	28.00
8.	Donald Brighton, University of Idaho	28.50
9.	James A. Brotsos, University of Illinois	29.17
Tie 10.	George L. Weir, Iowa State College	29.50
Tie 10.	Duane D. Walter, State College of Washington	29.50

Teams

1.	Iowa State College	82.50
2.	University of Connecticut	84.84
3.	Mississippi State College	93.18
4.	State College of Washington	93.67

5.	University of Massachusetts	95.00
6.	University of Idaho	95.67
7.	University of Minnesota	95.69
8.	Michigan State College	97.17
9.	Kansas State College	99.85
10.	Ohio State University	100.34

MILK

Individuals

1.	James Howard Sherrod, Kansas State College	12.25
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3.	Robert K. Wight, Iowa State College	15.67
4.	Russell J. Moe, University of Minnesota	15.92
5.	Richard Jackson Stanley, Mississippi State College	16.42
6.	Dee R. Morgan, Utah State Agricultural College	18.75
7.	James Warren Newell, University of Nebraska	19.25
8.	Harold A. Ramsey, Kansas State College	19.42
9.	William C. Coker, A & M College of Texas	19.75
10.	Donald E. Miller, University of Illinois	19.79

Teams

1.	Kansas State College	52.59
2.	Iowa State College	59.97
3.	Mississippi State College	64.67
4.	State College of Washington	66.42
5.	Agricultural & Mechanical College of Texas	68.42
6.	Utah State Agricultural College	70.00
7.	Ohio State University	70.50
8.	University of Illinois	70.79
9.	University of Connecticut	70.92
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ABSTRACTS OF LITERATURE

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and the Milk Industry Foundation

BOOK REVIEW

888. Methods of vitamin assay. Assoc. of Vitamin Chemists, Inc., Interscience Publishers, Inc., N. Y. 189 pp. 1947.

This volume is based upon actual laboratory check of methods for Vitamin A, carotene, thiamin, riboflavin, niacin and ascorbic acid. Chemical formulae, spectroscopic characteristics (except for niacin and ascorbic acid), sources, relative precision of methods and detailed methods of assay are presented for the vitamins listed above. Literature references are presented for assay of vitamins, D, E and K and for biotin, folic acid, *p*-amino benzoic acid, inositol, choline, pantothenic acid and pyridoxine. An excellent section is presented relative to sampling, though unfortunately, dairy products are slighted in this section. The volume is written critically and should be in the libraries of all who are engaged in vitamin work. E. W. Bird

ANIMAL DISEASES

W. D. POUNDEN, SECTION EDITOR

889. Incidence of Q fever in eastern Washington. R. DODDANANJAYYA, State College of Wash., Pullman. Pub. Health Repts., **64,** 39: 1230–1236. Sept. 30, 1949.

A seriological survey showed that Q fever exists in eastern Washington in both humans and animals. Six of 289 samples of human sera were found to have Q fever complemented-fixing antibodies, in titers ranging from 1:8 to 1:128. Three of these positive sera were from humans having close contact with animals while the other 3 persons had no occupational contact with animals. Out of 327 samples of blood sera from beef and dairy cattle, 9 were found to be positive with titers ranging as high as 1:128. The breed, age and sex of the animals had no special significance. D. D. Deane

BUTTER

O. F. HUNZIKER, SECTION EDITOR

890. Smörets struktur vid vanlig och kontinuerlig smörtillverkning. (The structure of butter by the usual buttermaking process as compared with the continuous method.) N. KING. Svenska Mejeritidningen, **40,** 9: 95–97. Feb., 1948. **40,** 10: 105–109. March, 1948.

The effect of physical and chemical factors on the structure and consistency of butter is discussed in detail. The microscopic structure of butter consists of fat globules, fat crystals, brine droplets and air cells. Liquid butter fat serves as embedding medium for these elements. Fe and Cu present in cream in combination with phospholipids and proteins are believed to be transferred to the butter. The number of fat crystals in free fat and their size are believed to play an important part in the hardness and consistency of butter. The size and number of brine droplets may have some influence upon the appearance and keeping quality of butter.

In the phase inversion of the cream as it occurs in the Alfa process for continuous buttermaking the following steps are enumerated: (a) fat globules distributed in milk serum, (b) beginning clumping with fat globules partly combined in clumps, (c) very large clumps with an irregular edge, (d) double emulsions in which both brine droplets, containing larger or smaller numbers of fat globules are present in the fat phase and fat globules with a birefringent peripheral layer and (e) a system with fat globules and brine droplets in free fat and no fat globules in brine droplets.

It is pointed out that microscopic methods are of considerable value in research involving butter structure but the microscopic method is useful only when dimensions are not smaller than 0.5–0.1μ. Undoubtedly there are present in butter, particles varying from 0.1μ to 1mμ such as small fat crystals in free fat, water veins and phospholi-

pid-protein membranes of fat globules. In order to obtain more information regarding these, it will be necessary to use methods that will be as satisfactory in the submicroscopic field as the microscopic methods are in the microscopic field.
G. H. Wilster

891. Metallic churns and butter kneaders having no kneading rollers. F. J. J. J. Henrard. (Assignor to Ecremeuses Melotte.) U. S. Patent 2,481,842. 2 claims. Sept. 13, 1949. Official Gaz. U. S. Pat. Office, **626,** 2: 497. 1949.

This metallic churn and butter kneader rotates on its horizontal axis, the 2 end walls being flat and parallel. Instead of the usual cylindrical shape, with the cross-section in the shape of circle, the cross-section of this churn is in the shape of 3 equal cyloidal curves, thus providing suitable agitation for churning and working action for the butter as the churn is rotated.
R. Whitaker

CHEESE

A. C. DAHLBERG, SECTION EDITOR

892. Cheese cutting machine. L. M. Seely. U. S. Patent 2,481,162. 3 claims. Sept. 6, 1949. Official Gaz. U. S. Pat. Office, **626,** 1: 203. 1949.

Cheese may be sliced in any shape by this vertical gravity-operated knife. A guard prevents operator injury by the knife. R. Whitaker

893. Rotary drum cheese grater. J. Orlando. U. S. Patent 2,481,336. 1 claim. Sept. 6, 1949. Official Gaz. U. S. Pat. Office, **626,** 1: 248. 1949.

A rotating drum having a rough surface grates the cheese as it is fed continuously to the drum.
R. Whitaker

894. Manufacture of rennet paste. E. C. Scott and G. W. McDonald. (Assignors to Swift and Co.) U. S. Patent 2,482,520. 4 claims. Sept. 20, 1949. Official Gaz. U. S. Pat. Office, **626,** 3: 809. 1949.

A paste is made of 60 parts whole milk curd and 1 to 12 parts of rennet extract and the pH adjusted to 4 to 5. R. Whitaker

Also see abs. no. 897.

DAIRY BACTERIOLOGY

P. R. ELLIKER, SECTION EDITOR

895. Mechanism of the fermentation of lactose by yeasts. Morrison Rogosa, U. S. Dept. of Agr., Washington, D. C. J. Biol. Chem., **175,** 1: 413–423. Aug., 1948.

Evidence is given that enzymatic hydrolysis of lactose to the component monosaccharides is not necessary for fermentation. Ten different lactose-fermenting organisms were used in the study.
A. O. Call

DAIRY CHEMISTRY

H. H. SOMMER, SECTION EDITOR

896. Methods for determining the iron content of milk. F. A. Johnston, Naomi Gellman and Juniata Strom, Cornell Univ., Ithaca, N. Y. J. Biol. Chem., **175,** 1: 343–347. Aug., 1948.

In 1943 the National Research Council reported the Fe content of milk as 2.0 mg./kg. This figure was lowered to 0.7 mg./kg. in their 1945 published tables. In 1944 the senior author reported a value of 0.32 mg. Fe/kg. A recheck of the dry ash method originally used showed it to be reliable when compared with two other methods. Ten separate milk samples from 5 distributors (presumably from the Ithaca area) were tested for Fe by 3 methods. The average values by each method were 0.38, 0.39 and 0.33 mg./kg. The highest reported value of any sample by any method was 0.59, and the lowest 0.25 mg./kg. Details of an improved wet ashing procedure are given. A. O. Call

897. The estimation of fatty acids of intermediate chain length by partition chromatography. M. H. Peterson and M. J. Johnson, Univ. of Wis., Madison. J. Biol. Chem., **174,** 3: 775–789. July, 1948.

While investigating the role of fatty acids in Cheddar cheese flavor a chromatographic method for the quantitative estimation of formic, acetic, propionic, n-butyric, caproic, caprylic and capric acids was developed. A detailed description for the preparation of both macro and micro chromatogram tubes and their development, as well as the titration of aliquots of the effluents is given. Sulfuric acid (27 to 35 *N*) was used as the nonmobile phase and Celite 545 as an inert filler. For routine estimations in biological materials one macro and three micro columns were used. Analyses of known mixtures, as well as butterfat with added known amounts of fatty acids showed recoveries with an 8% maximum error.
A. O. Call

898. Stable fortified milk products and process of preparing same. G. E. Grindrod. (Assignor

to Wis. Alumni Research Foundation) U. S. Patent 2,481,414. 11 claims. Sept. 6, 1949. Official Gaz. U. S. Pat. Office, **626,** 1: 268. 1949.

An insoluble salt of Cu, Fe or Mn is allowed to absorb an edible protective colloid and the dispersion is incorporated into liquid milk products to form a fortified concentrated sterilized food. R. Whitaker

899. Stable milk product containing added anti-anemia factor and process of making same. G. E. GRINDROD. (Assignor to Wis. Alumni Research Foundation.) U. S. Patent 2,481,415. 7 claims. Sept. 6, 1949. Official Gaz. U. S. Pat. Office, **626,** 1: 268. 1949.

Essentially the same as Abstract 898, except that ascorbic acid is added to the product before canning and sterilizing. R. Whitaker

DAIRY ENGINEERING

A. W. FARRALL, SECTION EDITOR

900. Practical ammonia refrigeration. Cooling buttermilk, condensed milk and ice cream mix. C. H. MINSTER, Greenbriar Dairy Products Co., Beckley, W. Va. Ice Cream Rev., **33,** 2: 52, 54, 56, 57, 58, 60. Sept., 1949.

The refrigeration load as applied to cooling buttermilk involves cooling the fluid skim milk from the pasteurization temperature of 180–190° F. to the setting temperature of 70–74° F., and cooling of the finished product from the setting temperature down to 50° F. or below. Surface coolers, plate-type coolers or coil vats may be used in cooling the fluid skim milk. Vat cooling with sweet water or brine is the most common method employed in cooling buttermilk from the setting temperature to 50° F. or below. Freezing of any part of the buttermilk and excessive agitation during cooling are to be avoided.

Sweetened condensed milk may be cooled in a coil vat or by an internal tubular cooler. The latter provides for continuous cooling from the pan with no intermediate crystallization period required. The well water temperature and the final temperature desired are variables which will affect materially the refrigeration load in cooling sweetened condensed milk.

Plain condensed milk may be cooled with a vat or plate-type cooler. The latter method appears to be growing more popular. Precautions should be taken to avoid contamination of the product and to prevent the incorporation of air during the cooling process. Evaporated milk, unless it is to be stored over night, need not be cooled prior to canning and sterilization.

Ice cream mix, because of its greater viscosity, which interferes with heat transfer, is more difficult to cool than fluid milk. To cool ice cream mix efficiently, either the surface area of the cooler must be increased or the time allowed for cooling must be increased. Regeneration has only limited application in cooling ice cream mix, since the sugar and stabilizer must be dissolved in the mix prior to pasteurization.

Data presented show that if an ice bank storage system is used, the hourly refrigeration load may be reduced by as much as 90% as compared with the use of direct expansion. W. J. Caulfield

901. Milk cooler. G. R. DUNCAN. U. S. Patent 2,482,579. 6 claims. Sept. 20, 1949. Official Gaz. U. S. Pat. Office, **626,** 3: 826. 1949.

This tank for cooling milk in cans is characterized by having a side door for easy insertion of the cans. A cooling coil below the can-supporting frame provides chilled water which is circulated and sprayed over the cans in the tank. R. Whitaker

902. Ice cream package filling mechanism. F. D. PALMER. U. S. Patent 2,482,593. 1 claim. Sept. 20, 1949. Official Gaz. U. S. Pat. Office, **626,** 3: 829. 1949

A sleeve slides in and out of the end of the tube leading from a continuous freezer. When the sleeve is projected, the valve on the end of the sleeve is closed and it is open when retracted, thus alternately filling and discharging a given portion of ice cream as the sleeve is moved up and down. R. Whitaker

903. Method of and apparatus for dehydrating liquid products. J. M. HALL. (Assignor to Drying and Concentrating Co.) U. S. Patent 2,481,418. 14 claims. Sept. 6, 1949. Official Gaz. U. S. Pat. Office, **626,** 1: 269. 1949.

Milk is heated rapidly under pressure to temperatures sufficient to destroy instantly bacteria and enzymes and immediately sprayed into a blast of air at a lower pressure to reduce the moisture content of the milk. R. Whitaker

904. Centrifugal separator with movably supported supply can therefor. W. H. HARSTICK and O. E. HEINTZ. (Assignors to International Harvestor Co.) U. S. Patent 2,482,272. 6 claims.

Sept. 20, 1949. Official Gaz. U. S. Pat. Office, **626,** 3: 745. 1949.

The supply tank which feeds this separator may be raised and lowered by means of a foot pedal at the base of the machine. When lowered, the supply tank outlet is positioned correctly for feeding directly into the spinning bowl.
R. Whitaker

905. Centrifugal homogenizer. H. Becchia. U. S. Patent 2,482,235. 2 claims. Sept. 20, 1949. Official Gaz. U. S. Pat. Office, **626,** 3: 735. 1949.

The fluid to be homogenized is introduced through a pipe into a rapidly rotating horizontal drum from which it is discharged rapidly by centrifugal force through small perforations in the outer wall of the drum, to impinge on serrations mounted close to the rotating drum. The homogenized product drains into a bowl-like vessel below the spinning drum and is discharged through an outlet in bottom. R. Whitaker

906. Dump can. V. Schwarzkopf. (Assignor to Lathrop Paulson Co.) U. S. Patent 2,480,778. 3 claims. Aug. 30, 1949. Official Gaz. U. S. Pat. Office, **625,** 5: 1415. 1949.

The features of this easily cleaned dump tank for milk are a simple means of preventing splashing when cans of milk are rapidly emptied into it and the inclusion of a removable strainer tray.
R. Whitaker

907. Building heating. J. C. McCabe, McGraw-Hill, New York, N. Y. Operating Engineer, **2,** 9: 19–34. Sept., 1949.

Topics covered in this review are behavior of heat, calculation of heat loads, infiltration, distribution of heat, distribution systems, heating units and auxiliaries. There are 81 tables and illustrations. Some of the heat distributors illustrated and discussed are radiators, wall radiators, convectors, baseboard heaters, panel heaters, blast coils, unit ventilators and propellor-fan units. The discussion of auxiliaries concerns valves, fittings and traps. H. L. Mitten, Jr.
Also see abs. no. 891.

DAIRY PLANT MANAGEMENT AND ECONOMICS

L. C. THOMSEN, SECTION EDITOR

908. Keep out of the hole with cost control. F. Merish. Milk Plant Monthly, **38,** 9: 34–36. Sept., 1949.

The 14 rules discussed for maintaining an effective cost control in the dairy plant are: (a) accurate accounting systems, (b) monthly profit and loss statements, (c) analysis of business figures, (d) comparative analysis by percentages, (e) depreciation in cost allotments, (f) overhead expense, (g) promotional outlay increases, (h) equipment modernization, (i) provisions for personal salary, (j) production cost knowledge, (k) departmentization of cost and sale figures, (l) insurance costs, (m) delinquent accounts and (n) improved supervision. J. A. Meiser, Jr.

909. Separate cabinets for "Take Home" sales build volume at drug stores. V. M. Rabuffo. Ice Cream Trade J., **45,** 9: 28, 31, 65–69. Sept., 1949.

The installation of separate serve-yourself cabinets for dispensing packaged ice cream may save drug stores their "take home" ice cream market. This would simplify proper pricing, eliminate waiting on rushed fountain clerks and, with carry-home provisions and proper location in the store, would increase sales and retain ice cream customers who rapidly are turning from drug stores to other retail outlets. W. H. Martin

910. Bonus plan spurs sales. P. L. Anders. Milk Plant Monthly, **38,** 9: 68–69. Sept., 1949.

Using the previous month's sales as the base period, increased sales of 1 to 3 points netted the routeman $1.00 per point. Four to five points obtained $1.50 each, whereas 6 points returned $2.00 per point. Besides being used for milk sales, this plan was used also for increasing buttermilk and cheese sales. J. A. Maiser, Jr.

911. Collecting "Slow accounts". L. Fanald. Milk Plant Monthly, **38,** 9: 38–39. Sept., 1949.

A drastic reduction in slow accounts was accomplished by the issuing of "nudge cards" to routemen whose duty was to attempt a collection each time they found a delinquent customer home. If, at the month's end, payments had not been made, red stickers were pasted on the face of the bill demanding immediate payment and these in turn delivered by the routeman to the customer. J. A. Meiser, Jr.

912. What it costs to serve dealers' customers of different sizes. P. P. Miller, General Ice Cream Corp., Schenectady, N. Y. Ice Cream Trade J., **45,** 10: 60. Oct., 1949.

The cost per gallon to serve customers of one of General Ice Cream Co.'s plants ranged from $0.2757 for customers of the 6001 to 7000 gal. group to $0.9035 for the 101 to 200 gal. group. These figures include delivery, selling and administrative expenses. To make the same profit per gallon on large and small accounts, the price to the small account may have to be very high.

W. H. Martin

FEEDS AND FEEDING

W. A. KING, SECTION EDITOR

913. Voederproeven over de invloed van organische zuren (vooral in verband met het aanzuren van ondermelk). (Feeding trials on the influence of organic acids, especially in relation to the acidifying of skimmilk.) (English summary.) TH. J. DEMAN, Institute for Modern Cattlefeeding, "De Schothorst", Hoogland near Amersfoort, Holland. Publication of the Nationale Coöperative Aan-en Verkoopvereniging voor de Landbouw (National Cooperative Buying and Selling Association for Agriculture.) "Centraal Bureau" G. A., Rotterdam, Holland. 31 pp. 1949.

Skimmilk, used in practice for fattening pigs and raising calves, often gave bad results caused by putrefying bacteria present in skimmilk of inferior quality. This can be improved by using the ripened product. Experiments were performed in fattening pigs with skimmilk acidified with lactic acid, acetic acid, formic acid and citric acid to a pH of about 5.7, and compared with culture-ripened product and sweet skimmilk. The artificially acidified skimmilk caused approximately the same rate of growth and food consumption as the culture-ripened product. Best results were obtained with the sweet skimmilk which was of good quality. With citric acid a somewhat retarded growth and a disturbance in the locomotion of the animals were observed. For practice, formic acid is most efficient, being cheap and having a low equivalent weight.

A growing experiment with calves, comparing skimmilk acidified with formic acid and culture-ripened skimmilk showed the formic acid product as favorable as the other one. An experiment on the influence of addition of 0.1% of citric acid to the fattening mash of pigs resulted, at times, in disturbances in the locomotion and in other cases in cannibalism (biting off each other's tails). Possibly citric acid works in this way via disturbance of the microflora in the intestines, influencing the production of B vitamins. The observed abnormalities, curable with yeast, may be caused by shortage of riboflavin and pantothenic acid, but this explanation needs further investigation.

A. F. Tamsma

GENETICS AND BREEDING

N. L. VAN DEMARK, SECTION EDITOR

914. Inheritance of a Karakul-type curliness in the hair of Ayrshire cattle. F. E. ELDRIDGE, F. W. ATKESON and H. L. IBSEN, Kansas State College. J. Heredity, **40**, 8: 205–214. Aug., 1949.

The Karakul-type of curliness involving irregularity in the diameter of the hair rather than uniform flatness was found in an Ayrshire herd. The curliness is pronounced at birth, resembling a newborn Karakul lamb, and becomes less curly with age. The seasonal variation noted was concluded to be caused by the shorter hair in summer when the curliness is less evident, as compared to winter when the curliness is characteristic. There is no sex difference in the expression of the responsible gene(s). The character was concluded to be due to a single autosomal dominant gene, *K*, and differs from the more common, variable type of curliness found in individuals of most breeds, and from the type of curliness associated with semi-hairlessness. L. O. Gilmore

HERD MANAGEMENT

H. A. HERMAN, SECTION EDITOR

915. Field tests of insecticides and spraying methods to control horn flies in dairy herds. W. S. MCGREGOR, U.S.D.A., Agr. Research Adm., Bureau of Entomology and Plant Quarantine. J. Econ. Entomol., **42**, 4: 641–643. Aug., 1949.

DDT, DDD, methoxychlor and toxaphene emulsions, each containing 0.5% of the toxicant, were compared for horn fly control in field tests on Jersey cattle in Texas. Spray application was made on cattle confined in stanchions. A nozzle pressure of about 200 lb./in.^{2} was used. Treatments began in June, when fly population averaged 25 or more per animal in every herd, and were repeated each time the population rebuilt to 25 per animal. Area of cattle treated and quantity of spray varied as follows: (a) 2 qt. on entire body, (b) 1 qt. on entire body, (c) 1 qt. on top line and (d) 1 qt. on underline.

There was great variation in range of pro-

tection periods against flies as afforded by insecticides. One qt. of any spray applied on the top line was as good as 1 or 2 qt. on the entire body. Underline treatment was less effective, except with toxaphene. E. H. Fisher

916. The DDT content of milk from a cow sprayed with DDT. R. H. Carter and H. D. Mann, U.S.D.A., Agr. Research Adm., Bureau of Entomology and Plant Quarantine. J. Econ. Entomol., **42,** 4: 708. Aug., 1949.

A high pressure sprayer was used to apply a wettable powder suspension at a concentration of 4 lb. of DDT/100 gal. of water. A Shorthorn herd was sprayed over the entire body to the point of super saturation. Milk samples were taken from 1 cow for DDT analysis. Milking was by hand, and no precautions to prevent contamination of milk with DDT were taken. No check sample of milk was taken before spraying. Beginning 2 d. after spraying, and at 5 subsequent irregular intervals, milk samples showed a range of from 3.0 to 0.4 p.p.m. of DDT over a period of about 5 wk.; the average was 1.3 p.p.m.

E. H. Fisher

917. Cows heat house. N. Holmquist, Swedish Government Research Institute for Farm Buildings, Lund, Sweden. Agr. Eng., **30,** 9: 425. Sept., 1949.

An average milking cow produces about 20,000 cal./d., 25% of which is latent in respiratory moisture. All of the latent and 60% of the sensible heat is wasted by being carried away by ventilating currents without heating the barn. The remaining heat is sufficient for keeping the temperature high enough.

The Swedish project attempts to use the waste heat from the barn for heating a 5-room house. A heat pump with its evaporator located in the barn's outgoing ventilating duct is used. The lowest design temp. for which the system is calculated is 5° F. The system with 10 to 15 milking cows will heat the house with no insulation on the house or barn. When heating oil is $45 per ton and electricity is 2¢/kw.-hr., the cost of heating is $245 with oil and $190 with the heat pump. The system was tried in sweden last winter and found to be practical.

H. L. Mitten, Jr.

918. Portable milker. A. I. Tupening. (Assignor to DeLaval Separator Co.) U.S. Patent 2,482,602. 5 claims. Sept. 20, 1949. Official Gaz. U.S. Pat. Office, **626,** 3: 831. 1949.

A milking machine, complete with vacuum pump, is mounted on a 2-wheeled cart.

R. Whitaker

ICE CREAM

C. D. DAHLE, SECTION EDITOR

919. Use of whey in sherbets. F. E. Potter and D. H. Williams, Agricultural Research Administration, USDA. Ice Cream Trade J., **45,** 9: 54–55, 86–88. Sept., 1949.

Approximately 75% of the 12 billion pounds of cheese whey produced annually is used as animal feed or wasted. Ransdell and Webb developed a sweetened condensed whey to overcome the high water content and perishable nature.

A good quality sherbet was made by using whey solids instead of milk solids. These sherbets contained 5% whey solids when made from plain condensed, sweetened condensed, or dehydrated whey but after adding sugar, stabilizer, flavor and citric acid to the fresh, separated whey, the whey solids content of the finished product was between 4 and 5%.

The whey sherbets were frozen most successfully in a continuous freezer. When a batch freezer was used excessive whipping occurred. Overrun in the batch freezer could be controlled by the addition of fat. A normal amount of citric acid was required for sherbet made from low acid whey, but no citric acid was needed with cottage cheese whey. The whey sherbets had a smooth body and texture, were more refreshing than other sherbets and had no whey flavor when made from a good quality whey.

W. H. Martin

920. Shrinkage. J. C. Lando and C. D. Dahle, Penn State College, State College. Ice Cream Trade J., **45,** 10: 90, 114–115. Oct., 1949.

Of 12 commercially shrunken samples of ice cream studied, all but 2 showed a formol titration in excess of the titration for the control mix of the same nitrogen content, indicating that the shrunken ice cream protein had undergone some degree of change causing them to have a higher formol titration. A study was made of changes in the distribution of mix proteins when treated with the proteolytic enzyme, trypsin. Mix treated with 2 g. of trypsin to 45 lb. of mix and held at

40° F. for 24, 48, 72 hr. showed little or no shrinkage in the resulting ice cream after 1 wk. in a cabinet at −15° F. Shrinkage did result when the mix was held for 96 hr., indicating that some change was occurring during the 72 to 96 hr. period which was of major importance in enzymatic shrinkage.

Other tests were made to determine any change in protein distribution when ice cream was subjected to severe dry ice exposure, and also the effect of the albumen-globulin fraction of milk protein in ice cream shrinkage. Additions of the unaltered whey proteins to ice cream mixes reduced shrinkage considerably. W. H. Martin

921. Control of ice cream texture with microscope. W. S. Arbuckle, Univ. of Md., College Park. Ice Cream Trade J., **45,** 10: 86, 114. Oct., 1949.

The microscopical examination of ice cream may reveal body and texture characteristics which are not readily detected organoleptically. Smooth textured ice cream will have a large number of evenly distributed ice crystals and air cells. Coarse textured ice cream will have numerous large ice crystals along with fewer small crystals and less uniformity of crystals and air cells.

Microscopic examination is made by preparing a thin section of ice cream, imbedding the section in immersion oil and examining at a magnification of 100 times. This work usually is done at hardening room temperature. W. H. Martin

922. New frozen citrus purees and their uses. E. A. Beavens, Bureau of Agr. and Ind. Chem., U.S.D.A., Pasadena, Cal. Ice Cream Trade J., **45,** 10: 58, 96. Oct., 1949.

Successful processing of citrus fruit purees has been accomplished and provides fruit bases which possess natural flavor, color and nutritive value. These purees can be kept in good condition for a year when stored at 0° F. Use 14 to 18 oz. of 5 to 1 orange puree and 1.5 oz. of a 50% solution of citric acid solution to 1 gal. of sherbet mix, stir thoroughly, freeze at 50 to 65% overrun and then place in containers and harden. For lemon sherbet only 10 to 14 oz. of puree and 0.5 oz. of 50% citric acid are needed. The citric acid solution should be added after freezing when the sherbet mix contains any milk products to prevent curdling.

Milk sherbet mixes should contain 2.5% fat, 2.5% milk solids and 25% sugar. W. H. Martin

923. Gallonage analysis. Anonymous. Ice Cream Trade J., **45,** 9: 42, 88–89. Sept., 1949.

The International Association of Ice Cream Manufacturers has compiled an analysis of U. S. wholesale and retail ice cream production by manufacturers. The recently issued Ice Cream Sales Index for 1948 shows the number of wholesale ice cream manufacturers in the U. S. to be 3,766, producing 91.7% of all commercial ice cream. Of these 3,766, there are 2,879 which sell ice cream by wholesale only, with the remaining 887 retailing in their own stores, though they are primarily wholesalers. The 10,394 manufacturers who made and sold at retail only accounted for only 8.3% of the national production.

Of the industry's 629,090,000 gal. produced, the 3,766 wholesale manufacturers were responsible for 577,026,000 gal. and the 10,394 retailers for 52,064,000 gal. This report is substantiated by figures from the U. S. Department of Agriculture based on 1946 operation and by the U. S. Census of Manufacturers' report for 1947.

W. H. Martin

Also see abs. no. 900, 902, 909, 912.

PHYSIOLOGY AND ENDOCRINOLOGY

R. P. REECE, SECTION EDITOR

924. The fate of radioactive copper administered to the bovine. C. L. Comar, G. K. Davis and Leon Singer, Fla. Agr. Expt. Station, Gainesville. J. Biol. Chem., **174,** 3: 905-914. July, 1948.

Fifteen cattle were given an isotope of copper (Cu^{64}), orally in some cases and by jugular injection in others. The Cu retention was very low when fed but was highly retained when injected intravenously. In both cases, the Cu retained was widely distributed in the body tissues but more concentrated in the liver. A table showing the Cu distribution in the various body tissues is included. A. O. Call

925. The transfer of immunity to the new-born calf from colostrum. E. L. Smith and August Holm, E. R. Squibb & Sons, New Brunswick, N. J. J. Biol. Chem., **175,** 1: 349–357. Aug., 1948.

Electrophoretic studies show that γ-globulin and T-globulin, both found in the serum of the mother, are not present in the serum of the new-

born calf or the new-born lamb, demonstrating that there is no placental transfer of antibodies in these species. This is in direct contrast to what is found in humans, where the γ-globulin in the serum of the new-born exceeds that of the mother. The calf acquires immunity through the igestion of colostrum. A. O Call

926. Passage of selenium through the mammary glands of the white rat and the distribution of selenium in the milk proteins after subcutaneous injection of sodium selenate. K. P. McConnell, Univ. of Rochester, Rochester, N. Y. J. Biol. Chem., **173,** 2: 653–657. April, 1948.

Radioactive selenium was injected subcutaneously into lactating white rats and was shown to be present in the carcasses of the suckling pups within 24 hr., thus confirming previous reports of selenium being transmitted in the milk. To determine the milk fraction carrying the selenium, the stomach contents of 2 litters of rats were removed and the milk curd resuspended and fractionated. The protein fraction carried selenium. A. O. Call

927. Effect of the blood glucose level on the secretion of the adrenal cortex. G. L. Steeples and H. Jensen, Medical Dep't., Field Research Laboratory, Ft. Knox, Ky. Am. J. Physiol., **157,** 3: 418–421. June, 1949.

Studies were made on 225–280 g. rats. Adrenal glands were weighed, blood sugar measured and cholesterol content of the adrenal glands determined. Hyperglycemia, induced by glucose administrated orally in a 50% solution, inhibited hormone release from the adrenal cortex as measured by the cholesterol content of the adrenal cortex. With the same measurement, hypoglycemia induced by insulin injections stimulated hormone release from the adrenal cortex.

How the blood sugar level influences the secretion of adrenal cortical hormones is not definitely known, although a plausible explanation is that the blood sugar level affects the secretion of the pituitary adrenocorticotrophic hormone, which in turn regulates hormone secretion by the adrenal cortex. V. Hurst

SANITATION AND CLEANSING

K. G. WECKEL, SECTION EDITOR

928. Quaternary ammonium compounds in dairy sanitation. C. C. Prouty, Dept. of Dairy Husbandry, State College of Washington, Pullman. Milk Plant Monthly, **38,** 9: 46–48. Sept., 1949.

A discussion of present knowledge of Quaternary ammonium compounds is presented. The various phases covered are: (a) methods of determining germicidal efficiency, (b) physical reaction of bacteria in contact with quaternary compounds, (c) bacteriostatic action of the compounds, (d) the influence of pH, type of water and organic matter on the germicidal efficiency and (e) selective action on organisms.

J. A. Meiser, Jr.

929. Deposition of aerosol particles. A. H. Yeomans, E. E. Rogers and W. H. Ball, U.S. D.A., Agr. Research Adm., Bureau of Entomology and Plant Quarentine. J. Econ. Entomol., **42,** 4: 591–596. Aug., 1949.

Tests with DDT aerosols determined the proportional deposition of toxicant on horizontal and vertical surfaces. Comparisons were made on the basis of (a) toxicity to insects, (b) chemical analysis, (c) visual observation of dyed deposits and (d) no. and size of particles.

Aerosol application in still air, simulating that in closed buildings, showed very little or no deposition on walls or other vertical surfaces. The particles settled almost solely upon the top of horizontal surfaces. Chemical analysis recovery of DDT from wall panels was less than 1% as great as from floor panels. Data on aerosol deposition in moving air, 2 to 16 mi./hr. also were included. E. H. Fisher

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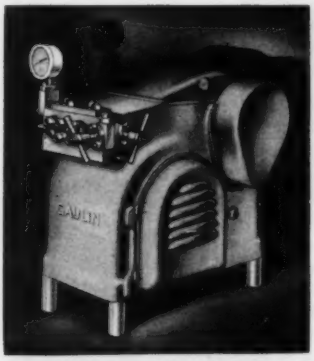

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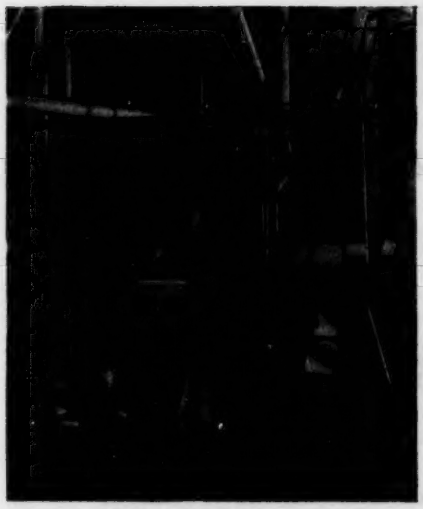

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